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&
LIGNARIA
HUNGARICA

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IN FOREST, WOOD
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Parasitoid complex of leaf miner *Phyllonorycter comparella* (Lepidoptera, Gracillariidae) in Hungary

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Abstract – Between 2011 and 2014, 1,154 mines of *Phyllonorycter comparella* (Duponchel) were collected at 12 locations in Hungary and were put into single-mine rearing containers. A total of 574 parasitoid specimens belonging to 29 parasitoid species (26 Chalcididae, 2 Encyrtidae and 1 Braconidae) emerged. Of these species, 13 have not yet been mentioned in either international or in Hungarian literature as a parasitoid of the *P. comparella*. The species assemblages of the parasitoid complexes varied greatly among the sample sites. The primary dominant species of the total samples was found to be *Sympiesis sericeicornis* (Nees), an abundant idiobiont solitary ectoparasitoid. Among the species reared, we have found specialist parasitoids such as *Achrysocharoides scaposa* (Erdős) and even species never recorded from *Populus* (*Zagrammosoma variegatum* (Masi)) according to the Universal Chalcidoidea Database.

***Phyllonorycter comparella* / *Populus alba* / parasitoid assemblages**

Kivonat – A *Populus alba*-n élő *Phyllonorycter comparella* (Lepidoptera: Gracillariidae) levélaknázó magyarországi parazitoid együttese. 1154 *Phyllonorycter comparella* (Duponchel) levélaknát gyűjtöttünk és tettünk egyedi nevelésbe 2011–2014 között, 12 Magyarországi helyszínről. A nevelésből kikelt 574 parazitoid egyed alapján 29 fajt (26 Chalcididae, 2 Encyrtidae és 1 Braconidae) sikerült azonosítani. Ezek közül 13 fajt sem a nemzetközi sem a hazai szakirodalom korábban nem említ, mint a *P. comparella* parazitoidjait. Különböző mintagyűjtési helyszínek parazitoid komplexum fajegyüttesében eltérések mutatkoztak. A teljes minta elsődleges domináns faja a *Sympiesis sericeicornis* (Nees), egy gyakori idiobiont szoliter ektoparazitoid volt. A nevelésből kikelt fajok között olyan specialista fajokat is azonosítottunk, mint az *Achrysocharoides scaposa* (Erdős) valamint *Populus*ról eddig, a Nemzetközi Chalcidoidea Adatbázis (Universal Chalcidoidea Database) által még nem regisztrált *Zagrammosoma variegatum* (Masi) parazitoid fajt is.

1 INTRODUCTION

Phyllonorycter comparella (Duponchel) is a palaearctic species occurring all over the Eurasian continent (De Prins – De Prins 2014). Its main host plant is *Populus alba* (Gozmány 1956), but it can also be found on *Populus tremula* (Linné) and *Populus × canescens* (Ation) (De Prins – De Prins 2014). It is a bivoltine species; the adults fly from June till August and

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also in October (Szócs 1977). The species overwinters in the adult stage. The blotch mines are located on the underside of the leaf between the leaf veins (Csóka 2003).

Based on our observations, *Ph. comparella* can locally occur at a very high density with several mines on a single leaf. Similar observations have been made by Yedfremova et al. in Russia (Ul'yanovsk Province) in 2007 (Yefremova et al. 2009). A number of papers predict a change in insect behaviour because of the effects of climate change (Cannon 1998, Csóka 1996, Csóka 1997, Dukes et al. 2009, Fleming – Volney 1995, Kirilenko – Sedjo 2007, Walther et al. 2002), and common and even rare species may become severe pests. Therefore, we believe that this species has the potential ability to cause severe outbreaks and possibly heavy damage on *Populus alba* and *Populus* × *canescens*.

It is always important to study the regulating potential and the species composition of the natural enemy complex of such a species.

The parasitoid complex of *Ph. comparella* has been inadequately studied thus far. Two of the main parasitoid databases publish a rather small number of parasitoids (De Prins – De Prins 2014, Noyes 2015). In Hungary only two researchers (Erdős 1956, Szócs 1965, Szócs 1979) reared parasitoids from *Ph. comparella*. The aim of this work is to summarize the knowledge of the parasitoid species developing in *Ph. comparella* in Hungary in comparison with the data available in European literature.

2 MATERIAL AND METHODS

We studied the parasitoid complexes of various leaf miners over a period of four years (2011–2014). A total of 1,154 leaf mines were collected from *P. alba* at 12 locations across Hungary (Table 1).

Single mine rearings were used. The mines were cut out from the leaf in order to avoid contamination from other parasitoids (i.e. egg or aphid parasitoids) which parasitize other insects on leaves. After a short drying period, the samples were placed individually into air ventilated plastic rearing tubes.

The parasitoids that emerged were preserved in 95% ethanol, and later identified by Csaba Thuróczy and George Melika. The emerging leaf miner adults were also identified to make sure that the identification of the leaf mines was correct. After the identification process, the adult insects were kept in alcohol for further molecular analysis.

3 RESULTS AND DISCUSSION

From the samples collected during the four consequent years (2011–2014), a total of 574 parasitoid specimens emerged from 255 parasitized samples. These specimens belong to 29 parasitoid species (26, 2, and 1 species to the families Chalcididae, Encyrtidae and Braconidae, respectively). The total parasitization rate of our samples was 22.1% (n=1154 samples collected), and at different sample locations (with regard to the sample size) varied between 7.3% (n=55) and 75% (n=4) (Table 1).

Table 1. A summary of the sampling locations, the sample sizes and the parasitism rates of our rearings.

WGS84: the coordinates of the sample plots;

TMC: total leaf mines collected; TPM: total parasitized leaf mines;

TSE: total specimen reared; P%: percentage of parasitism.

Localities	WGS84 (GPS) coordinates		Sampling date	TMC	TPM	TSE	P%
	Lat. (N)	Long. (E)					
Ásotthalom	46°11'26.90"	19°46'25.00"	07.10.2013	147	21	25	14.3
			07.15.2014	238	73	99	30.7
Gödöllő	47°36'06.70"	19°24'17.91"	07.27.2012	3	0	0	0
Gyöngyösoroszi	47°50'40.45"	19°54'03.54"	08.13.2012	5	0	0	0
Hort	47°40'52.51"	19°45'20.60"	09.08.2013	4	3	3	75
Jánd	48°06'56.28"	22°21'24.69"	06.19.2013	192	44	256	22.9
Kiskőrös	46°35'12.72"	19°15'18.75"	07.08.2013	17	9	19	52.9
Kiskunhalas	46°22'13.47"	19°34'03.04"	07.13.2011	45	17	39	37.8
			07.15.2014	72	12	11	16.7
Mátrafüred	47°49'31.36"	19°58'28.60"	06.07.2011	39	18	22	46.2
			06.28.2011	47	13	28	27.7
			06.26.2012	108	1	5	0.9
			06.25.2013	56	8	8	14.3
Poroszló	47°39'02.11"	20°40'25.28"	07.11.2013	43	8	16	18.6
Püspökladány	47°20'04.55"	21°05'22.83"	07.11.2013	55	12	19	21.8
Tatabánya	47°31'48.96"	18°25'42.11"	10.19.2013	55	4	7	7.3
Törtel	47°05'15.56"	19°53'24.33"	07.08.2013	28	14	17	50
TOTAL				1,154	257	574	22.3

The identified parasitoid species of the complex is listed in Table 2.

We compared our species list with two of the most important on-line databases, the Universal Chalcidoidea Database, hosted by the British Natural History Museum (Noyes 2015) and the Global Taxonomic Database of Gracillariidae (Lepidoptera), hosted by the Belgian Biodiversity Platform (De Prins – De Prins 2014) and discovered that 15 species found during this research were not listed in these databases as parasitoids of the *Ph. comparella*.

From the samples collected at Ásotthalom, a total of 124 specimens belonging to 14 species have been identified. The parasitization rate at this place was 24.2% with 93 parasitized samples. The parasitoid complex was dominated by the synovigenic idiobiont solitary *Sympiesis sericeicornis* (Nees, 1834) (43%, n = 93 parasitised samples) and *Zagrammosoma talitzkii* (Boucek, 1961) (12.9%, n=93). Subdominant species were *Pediobius saulius* (Walker, 1839) (8.6%), *Minotetrastichus frontalis* (Nees, 1834) (8.6%), *Closterocerus trifasciatus* (Westwood, 1883) (7.5%) and a species belonging to the family Braconidae (7.5%). *S. sericeicornis* was also dominant at two other locations with small sample sizes (Kiskőrös and Törtel) and present in nearly all samples from the 12 sampling locations. *Agéniaspis testaceipes* (Ratzeburg, 1848), was the dominant species of the samples collected at Jánd (n = 192 total samples collected) causing 43.2% (from n = 44 parasitized samples) of the leaf miner mortality.

Table 2. The checklist and the number of parasitoid species reared from *Phyllonorycter comparella* in Hungary. Species marked with “*” have been recorded for the first time from *P. comparella*.

Parasitoids reared	Ásotthalom	Hort	Jánd	Kiskőrös	Kiskunhalas	Mátrafüred	Poroszló	Püspökladány	Tatabánya	Törtel	TOTAL
Encyrtidae											
<i>Ageniaspis testaceipes</i> *	23		230	11				2	4		270
<i>Encyrtidae</i> sp.*					23	24					47
Eulophidae											
<i>Achrysocharoides atilis</i> *						1					1
<i>Achrysocharoides cilla</i> *					1	15					16
<i>Achrysocharoides scaposa</i>				1		3	12	14		4	34
<i>Aprostocetus</i> sp.					1						1
<i>Baryscapus nigroviolaceus</i>	2		2								4
<i>Baryscapus</i> sp.					2	4					6
<i>Chrysocharis</i> sp.	2										2
<i>Chrysocharis laomedon</i>			3								3
<i>Chrysocharis nephereus</i>	1	1			1						3
<i>Chrysocharis pentheus</i> *	1					1					2
<i>Chrysocharis</i> sp. “A”*									1		1
<i>Cirrospilus elegantissimus</i>			4			2					6
<i>Cirrospilus lynceus</i>			1		1		1				3
<i>Closterocerus trifasciatus</i> *	8		5	1	9	1			1		25
<i>Minotetrastichus frontalis</i>	8		2				2				12
<i>Neochrysocharis formosus</i>	5	1									6
<i>Pediobius pyrgo</i> *					2						2
<i>Pediobius saulius</i>	9		2		2	1				1	15
<i>Pnigalio agraulis</i>			2								2
<i>Pnigalio pectinicornis</i>			1								1
<i>Pnigalio soemius</i> *	1										1
<i>Sympiesis acalle</i> *	1										1
<i>Sympiesis dolichogaster</i> *						1					1
<i>Sympiesis sericeicornis</i>	42		3	6	2	9	1	3	1	8	75
<i>Zagrammosoma talitzkii</i> *	13				6					4	23
<i>Zagrammosoma variegatum</i> *						1					1
Braconidae											
<i>Braconidae</i> sp.	8	1	1								10
TOTAL	124	3	256	19	50	63	16	19	7	17	574

From the 12 species that were present in these samples, we identified only one parasitoid species belonging to the Braconidae family. At this location, the sub dominant species were *C. trifasciatus* (11.4%, n=44 parasitized samples) and *Cirrospilus elegantissimus* (9.1%, n = 44 parasitized samples). *Zagrammosoma talitzkii* was dominant in the samples collected at Kiskunhalas, representing 21.4% of the 28 parasitized samples. The species occurred at only 3 locations. The secondary dominant species of the complex was *C. trifasciatus* (17.9%, 28 parasitized samples) and an unidentified species from the Encyrtidae family. *Achrysocharoides cilla* (Walker, 1839) was only dominant in the samples collected at Mátrafüred, being present in 32.5% of the 40 parasitised samples.

The species also occurred in the samples from Kiskunhalas with an insignificant parasitization rate. The secondary dominant species was *S. sericeicornis* (22.5%, n = 40 parasitized samples). *Achrysocharoides scaposa* (Erdős, 1961) was the dominant species at Poroszló (12.5%, n = 8 parasitized samples and 43 total samples collected) and Püspökladány (17.5%, n = 12 parasitized samples and 55 samples collected) but reared from a relatively small sample size. The species has been observed in samples from five different collecting locations.

The samples from Hort and Tatabánya contained few parasitized samples with only 3 parasitoid species emerged with one specimen each. *S. sericeicornis* is a solitary idiobiont species and was the most frequent species with a high capacity for controlling the miners. As with *C. trifasciatus*, it has a wide range of hosts and both species can be also be secondary parasitoids (Askew 1979, Stojankovic and Markovic 2005, Noyes 2015).

A more specialized species with a narrow host range, the koinobiont gregarious *A. testaceipes*, was also abundant, but dominant only at one sample place. According to the Universal Chalcidoidea Database (Noyes 2015) *A. scaposa* has been reared only from *Ph. comparella*. We identified the species in 5 of our samples and it was dominant in 2 (Püspökladány and Poroszló).

The gregarious koinobiont *A. cilla* was identified from only 2 sample locations (Kiskunhalas and Mátrafüred). Despite its low abundance within the samples, at Mátrafüred it seems to be the major controlling agent of its host. *Zagrammosoma variegatum* (Masi, 1907), an idiobiont solitary ectoparasitoid, has never been described from any species living on *Populus* sp (De Prins and De Prins 2014, Noyes 2015). In our research, we only observed the species at Mátrafüred. At this location we identified another rare and semi-specialist species, *Achrysocharoides altilis* (Delucchi, 1954).

In 3.5% of the 257 parasitized mines we found more than one species of coexisting parasitoids. On these rare occasions, both parasitoid species/specimens are able to complete the larval development. Such events/phenomena are termed the coexistence of multiple parasitoids, a phenomenon among parasitoids of various insects that is well known and has been documented for a long time (Askew – Shaw 1979, Amarasekare 2000a, Amarasekare 2000b, Klapwijk – Owent 2011, Borer et al. 2004, Hackett-Jones et al. 2009, László – Tóthmérész 2013). The coexistence of two parasitoids foraging on a single host occurs when the distribution of the parasitoids is sufficiently aggregated (May and Hussel 1981, Klopfer and Ives 1997), which triggers intra and interspecific competition (MacArthur and Levins 1967). The successful development of the parasitoids on a single host requires the partitioning or sharing of the food resource (Harvey et al. 2013) by partial host consumption (Miller 1982) and/or the separation of the temporal niche (Hackett-Jones et al. 2009, Amarasekare 2000a) In this case, the competition between the parasitoid species is delimited by the earlier mentioned requirements.

In our samples we registered 9 cases of multiple parasitism. In most cases (6 of 9) the parasitoid couple was composed from a koinobiont and an idiobiont parasitoid, where the koinobiont is a primary parasitoid and the idiobiont is a secondary generalist parasitoid. Therefore, we might say that it is likely that the first (primary) encounter could be the

koinobiont endoparasitoid. On one occasion, we registered two idiobiont ectoparasitoids that had completed their stages.

The most surprising finding was the presence of three koinobiont species from a single mine, which is a rather rare event (Askew 1979, Miller 1982).

Next follows a brief overview on the biology of the parasitoid pairs emerged from single mines (abbreviations: En = endoparasitoid; Ec = ectoparasitoid; G = gregarious; S = solitary; K = koinobiont; I = idiobiont. Read as follows: En,G,K = Endoparasitoid, Gregarious, Koinobiont):

- 1 female specimen of *Achrysocharoides altilis* (En,G,K) +
1 male specimen of *Baryscapus* sp. (Ec,G,I)
- 1 female specimen of *Braconidae* (En,S,K) +
1 male specimen of *Pnigalio agraulis* (Ec,S,I)
- 2 female specimens of *Achrysocharoides scaposa* (En,G,K) +
1 female and 1 male specimen of *Minotetrastichus frontalis* (Ec,G,I)
- 1 male specimen of *Baryscapus nigroviolaceus* (En,S,K) +
1 female specimen of *Minotetrastichus frontalis* (Ec,G,I)
- 1 female specimen of *Chrysocharis nephereus* (En,S,K) +
1 female specimen of *Pnigalio soemius* (Ec,S,I)
- 1 male specimen of *Pediobius saulius* (En,S,K) +
1 female specimen of *Zagrammosoma talitzkii* (Ec,S,I)
- 1 female specimen of *Braconidae* (En,S,K) +
1 female specimen of *Minotetrastichus frontalis* (Ec,G,I)
- 1 female specimen of *Zagrammosoma talitzkii* (Ec,S,I) +
1 male specimen of *Sympiesis sericeicornis* (Ec,S,I)
- 1 female specimen of *Chrysocharis nephereus* (En,S,K) +
1 female specimen of *Neochrysocharis formosus* (En,G,K) +
1 male specimen of *Braconidae* (En,S,K).

The two relevant online databases (Noyes 2015, De prins and De Prins 2014) list only a small number (19) of parasitoid species associated with the *P. comparella*, reflecting a small amount of studies regarding species load of the leaf miners parasitoid complexes. In this work we contribute 13 species of parasitoids as first records for the host *Phyllnorycter comparella*. Overall, we summarize that 36 parasitoid species have been recorded from *Ph. comparella* so far, including 29 species listed in the present publication. The species composition of the parasitoid complexes of sample sites varied greatly. It is acceptable to say that the parasitoid species of the leaf miner presented a habitat-specific occurrence as found by Hawkins (1994) and Gibbison et al. (1997). Common species, with a generally wide host range, being abundant in nearly all places represented the highest number of species of the entire complex (18 species). The endoparasitoid species, like the *A. testasceipes*, had the biggest impact on the leaf miner *P. comparella*.

As found at the sample sites, the *Ph. comparella* leaf miner can sometimes be very abundant (Yefremova 2009, personal observation). Climate change might trigger outbreaks of this species (as seen with other insects), causing heavy damage to its host plant (Kyle et al. 2014).

Nowadays many forests are composed from a low diversity of trees due to economic forest use; this low diversity of trees may help to intensify the outbreaks of many species (Jactel and Brockerhoff 2007). A number of papers show that diverse habitats contain more complex food-webs with a number of natural enemy species (Paine 1966, Polis and Strong 1996). It is always important to study the species composition of both native (Szócs et al. 2013) and invasive (Szócs et al. 2014) species with outbreak ability.

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Sex Ratio Analysis of Some Macrolepidoptera Species Collected by Hungarian Forestry Light Traps

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Abstract – We analysed the sex ratio of 32 macrolepidopteran species caught by Hungarian forestry light traps. That the ratio of males and females collected by light trap varies by species has been known for decades; however, the sex ratio found in the natural population is not known. All 32 species were processed separately, but by the same method. Both males and females were counted throughout the whole swarming. We calculated these figures and inspected the difference in the level of significance with the χ^2 test. For each swarming we calculated the percentage of males and females. We also calculated the values of the variation coefficients, which express the deviations in average percentages.

Males make up the majority of the moths captured in the trap; this result was mirrored by 29 of the 32 species investigated. One of the exceptions was the *Pelosia muscerda* Hfn. where we observed a male to female ratio that was equal. In addition to that *Watsoniana cultraria* Fabr. is the only one species captured by light traps that showed a significant female majority.

Our results confirm that the majority of moths captured in traps are males. However, the proportion of males and females of each species, and even within the same species, tended to differ greatly with each swarming. Yet, it must be noted that these results speak only for those specimens captured by light traps and cannot be related directly to the actual sex ratio of populations living in the natural environment.

Macrolepidoptera / sex ratio / forestry light-trap / Hungary

Kivonat – A magyar erdészeti fénycsapdák által gyűjtött néhány macrolepidoptera faj ivararányának elemzése. Évtizedek óta ismert, hogy fénycsapdával gyűjtött hímek és nőstények aránya fajonként változik. Nem ismert azonban, hogy milyen a nemek aránya a természetes populációban. 32 fénycsapdával gyűjtött Macrolepidoptera faj hím és nőstény arányát elemeztük. Minden fajt külön-külön dolgoztunk fel, de azonos módszerrel. Mind a hímeket, mind nőstényeket megszámláltuk egész rajzás során. Ezeket összeadtuk és az eltérések szignifikancia szintjét χ^2 próbával ellenőriztük. Kiszámítottuk a variációs koefficiens értékeket is, amelyek az eltéréseket az átlag százalékában fejezi ki.

Megállapítottuk, hogy a vizsgált 32 fajból 29 rajzásban magasabb volt a befogott hímek száma, mint a nőstényeké. Kivétel volt a hamvas zuzmósövény (*Pelosia muscerda* Hfn.), amelynél a hím és nőstény arány egyenlő és egyetlen faj bükkfa sarlósszövő (*Watsoniana cultraria* Fabr.), amelynél a nőstények voltak többségben.

Eredményeink szerint a legtöbb faj esetében több a fénycsapdával befogott hím mint nőstény, azonban ez az arány az egyes fajoknál, de még ugyanazon faj, más és más rajzásában is nagyon különböző. Ezek az eredmények azonban nem vonatkoznak közvetlenül a természetben élő populációk valós hím-nőstény arányaira, hanem csak a fénycsapdás fogásokra.

Macrolepidoptera / hím nőstény arány / erdészeti fénycsapda / Magyarország

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1 INTRODUCTION

That the proportion of males and females captured by light trap varies by species has been known for decades; however the sex ratio within the natural population is unknown.

Within the various orders of insects, Williams (1939) studied the sex ratio by species of the specimens caught. Of the 51 species of the Noctuidae family, the females of 2 species were not attracted to light at all. The females of 27 species represented 1–20% of the total number of specimens: this ratio was 20–46% with 16 species: the number of males and females was identical in the case of 3 species and there were only 3 species where more females than males were attracted to light. There is also a behavioural dimorphism: in 2 samples of 37 individuals of ghost moth (*Hepialus humuli* L.) only 22% males flew to light traps even though sweep net samples gave an approximately 50:50 sex-ratio (Williams 1939). According to Mallet (1984), in the case of *Hepialus humuli* L. more females than males are attracted to light, which suggests that the females are the more mobile gender.

Nanu – König (1968) examined 13 Lepidoptera families to establish the ratio of males and females attracted by light. The females of the various families represented 0.2–30% of the catch. Schurr (1971) captured mostly male specimens of the vine moth (*Eupoecilia ambiguella* Hbn.) with his trap running on white light (of 510–610 nm). Jáfás et al. (1974) used different sources of light to study the sex ratio of the turnip moth (*Agrotis segetum* Den. et Schiff.). Whatever the light source, the proportion of males was between 48–66%. Czencz (1973) holds that the males of the diamondback moth (*Plutella xylostella* L.) are more drawn to light than the females (75–25%). She does not provide any explanation for this, however.

Bürgés – Gál (1980), with a 125W mercury light trap, found that with the nut fruit tortrix (*Cydia splendana* Hbn.), males appear 3–4 days earlier than females. The male to female ratio is 1.9:1. This was almost the same as the male to female ratio of the European chestnut weevil (*Curculio elephas* Gyllenhal), which was 1.83:1.

Egyptian Bollworm (*Earias insulana* Boisduval) moths were collected by Yathom (1981) from mercury vapour light traps operating in Israel between 1974 and 1980. He concludes that the sex ratio generally favoured males.

El-Abdullah et al. (1984) report on a mere 10% of the trapped specimens of the Asiatic rice borer (*Cilo suppressalis* Walker) being males. Skuhravý et al. (1993) caught male specimens of the saddle gall midge (*Haplodiplosis marginata* von Roser) almost exclusively, both with a Minnesota-type and a UV light trap.

Itämies et al. (1986) employed a light trap to examine the flight pattern of grey mountain carpet (*Entephria caesiata* Den. et Schiff.) between 1978 and 1982 in the Finnish forest areas of Lapland. With the exception of 1978, males dominated the catch, although this dominance was not overwhelmingly high.

Some researchers have published statements of general validity. Novák (1974) has published sex index data relating to 96 species of moths trapped. Depending on the ratio of females, he arranged the species examined into five classes and, within certain limits, he regarded his results as constant. Malicky (1974) also believes that the sex ratio of a captured species is the same over many years through the use of the same source of light at different places of observation; in other words, sex ratio is value specific to a given species. Szarukán (1975) claims that the average proportion of female specimens of the dog's tooth (*Lacanobia suasa* Schiff.) is 33%. He found minimal difference between the first and second generations. A study in Egypt (Sadek 2001), found that 8.04% of the female mediterranean brocade (*Spodoptera littoralis* Fabr.) specimens that had been trapped had not been fertilized, while the ratios of those fertilized once or more than once were 37.25% and 54.76%. The sex ratio of the sulphur knapweed moth (*Agapeta zoegana* L.) was equal when examined at daytime, but at night the overwhelming majority of the specimens caught by light trap were male. The

difference may be explained by the dissimilar reaction of the two sexes to light (Story et al. 2001). In Switzerland Cordillot – Duelli (1986) noticed that the ratio of females captured by light trap in one generation of European corn-borer (*Ostrinia nubilalis* Hb.) was 47.3%. The above mentioned authors regard sex ratio as a static value specific to a species, despite the fact that the annual differences in sex ratio are valuable results related to population changes and not statistical deviation data without a biological value (Szeőke – Szarukán 1982). According to Mohai – Herczig (1979) and also to Lesznyák et al. (1993), the proportion in percentage of females rises and falls in harmony with the rise and fall of the amount of insects caught in light traps. The latter of the abovementioned authors have found that the ratio of females is affected first of all by the minimum values of temperature. El-Deeb (1992) established that the number of European corn-borer (*Ostrinia nubilalis* Hbn.) females captured by light trap surpassed that of the males. However, the ratio was affected positively by maximum and minimum temperature, and negatively by relative vapour content.

Some authors have found that both males and females have to be in a certain physiological state to be attracted to sources of light. Terskov – Kolomiec (1966) report that females of the white satin moth (*Leucoma salicis* L.) can be trapped before egg laying, while those of the gypsy moth (*Lymantria dispar* L.) in Siberia are more prone to be trapped after egg-laying. This latter bit of information is of special interest because there are no data in literature about how gypsy moth females of the European and American populations fly to light considering that the females there are incapable of flight. In contrast to that females of the gypsy moth (*Lymantria dispar* L.) population in East Asia do fly (Wallner et al. 1995; Reineke – Zebitz 1998; Charlton et al. 1999). Females of the European corn-borer (*Ostrinia nubilalis* Hbn.), on the other hand, may fly to light any time following their emergence from the pupal state (Showers et al. 1974). Then again, Elliott – Dirks (1979) claim they are trappable in the largest numbers 3.2 – 4.4 days after mating.

Sathiyandam – Baskaran (1999) observed that the ratio of females of the groundnut leaf miner (*Aproaerema modicella* Deventer) in India changed during different periods in the night. The ratio of mated to unmated females also changed. The number of males and females of some caddis fly (Trichoptera) species caught by light traps in New Zealand displayed a definite change in swarming. Males were the majority at the start of the swarming, while females made up the majority at the end (Ward et al. 1996). Dickler – Steuerwald (1997) found significant differences from one year to the next in the number of specimens as well as in the sex ratio of noctuids (Noctuidae) captured by light traps in apple orchards in Germany.

In the study of Myers et al. (1998) the sex ratio of gypsy moth (*Lymantria dispar* L.) pupae varied strikingly between low-density and high-density populations.

Altermatt et al. (2009) experimentally studied the flight to light behaviour of two moth species – the small ermine moth, *Yponomeuta cagnagella* (Hbn.), and the scorched carpet moth, *Ligdia adustata* (Den. et Schiff.). They found that male moths were significantly more (about 1.6 times more frequently) attracted to light than female moths. It was established that there is a sexual dimorphism in the flight to light behaviour of moths.

Garris – Snyder (2010) investigated and recorded the sex ratio of 28 southern species in the USA. They tested the well-known view that UV light-trap collections of moths are considerably skewed toward males. Twelve species demonstrated a statistically notable male preponderance, but a wide range of sex ratios was found. Two of the 28 species demonstrated significant bias for both males and females during different observation periods, illustrating the need to collect over the entire flight period. Since the sex ratio of collected organisms varies by species and by time, this knowledge must be taken into consideration when using light trap collection to make population estimates and to gather information for conservation or control of any particular species.

According to Tabadkani et al. (2013) appreciation of the proportion of genders of arthropods is a pertinent issue not only in ecological studies and in biological programs, but also in plant protection. In this study, they continued to examine the factors leading to erroneous estimates of sex ratios of insect species. They examined the predatory gall midge, *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae), and the results explicitly suggest that direct estimation of the sex ratio in natural populations may be affected by some secondary factors such as differential mortality of sexes, protandry, and differential distribution of males and females over time and/or across habitat.

While they could provide us with important, useful information for everyday practice, there are very few publications reporting on research of this kind. Unfortunately, this kind of research is both time consuming and energy consuming; thus, in the foreseeable future, we cannot expect any major breakthrough in this area.

Researchers have been studying the sex ratio of the insects trapped for decades, but their observations had little practical value for plant protection prognostics. The authors of most studies confine themselves to releasing the figures recording the number of males and females, perhaps even their ratio in terms of percentage of the captured specimens, but they refrain from drawing any conclusions. Admittedly, any such attempt would be a vain endeavour, especially in the case of the data provided by light traps operating for short periods. Whereas if the sex ratio of the populations in the environment shifts in the direction of a preponderance of females and that change is reflected in the catch, a growth in the number of the females trapped might indicate the start of gradation. Therefore, awareness of the regularity of sex ratio transformation may also be used for the purposes of prognosis in the case of species where both sexes fly well to light. Some researchers have published general statements concerning this.

When the sex ratio of an observed species at different observation sites and in different years produces decidedly different values, it would be useful to examine regularities in changes. From the point of view of prognosis, the differences in time have main importance, because they might be related to hypercyclic movement. Our own research revealed a rise of the number of turnip moth (*Agrotis segetum* Den. et Schiff.) females in the years which were followed by gradation (in years 1962 and 1968) (Nowinszky – Kiss 1981). It is assumed that the proportion of females affects the number of individuals of future generations. In the year prior to the years of gradation, the number of females increased.

We examined the changes of the number of the females in the turnip moth (*Agrotis segetum* Den. et Schiff.) population between 1957 and 1990. The light-trap catches of 65 observing stations were used (Kiss et al., 2003).

It is concluded that over 99 specimens in each generation the ratio of females is 0.38 from all individual number. If the number of individuals is between 5 and 99, the proportion is 0.44. The proportion of females is 0.46 if the number is between 100 and 499; it is 0.38 between 500 and 999. Between 100 and 999 this value is 0.40. For all observing stations, we calculated the female individual proportions and the 95% confidence intervals for them. We established that higher female proportion belongs to the lower individual numbers, but this proportion is close to the feature if individual numbers are high. It was observed that in the year before gradations begin, the rate of females was extremely high in many cases.

2 MATERIAL

The development of a light trap network began in 1952 in Hungary. The traps were used in research institutes, for plant protection, and for forestry purposes. The three-type light trap network is still working and works with uniformly Jermy-type traps. Over the past decades,

the national light-trap network has provided an enormous and inestimable amount of scientific insect material for entomological research and plant protection practice (Nowinszky 2003).

The Jermy-type light trap (Jermy 1961) consists of a frame, a truss, a cover, a light source, a funnel, and a killing device. All the components are painted black, except for the funnel, which is white. A metal ring holding the funnel and a zinc-plated tin joins the steel frame. The cover is 100 cm in diameter. The distance between the lower edge of the cover and the higher edge of the funnel is 20–30 cm. The light source is a 100W normal electric bulb with a colour temperature of 2900°K. The lamp is in the middle of the trussing, 200 cm above ground. The upper diameter of the funnel is 32 cm, while the lower one is 5 cm, and its height is 25 cm. In each case chloroform was used as a killing agent.

The forestry light traps are operational from 6 p.m. (UT) to 4 a.m. every night of the year, regardless of weather, or the time of sunrise and sunset. The operation is suspended only on days when the temperature is below 0 C° and the ground is covered by an unbroken layer of snow. All the insects trapped during the course of a night go into the same collecting jar and so a single set of data will represent the nightly catch result at the given observation site.

In this study we used the catch data of the Hungarian Forestry light trap network of the Forest Research Institute. The light traps were operating in 16 light trap stations across the whole territory of Hungary. The light trap stations, geographic coordinates and years of operation are presented in *Table 1*.

Table 1. The light trap stations, geographic coordinates and years of operation

<i>Light-trap stations</i>	<i>Geographic coordinates</i>	<i>Years of operation</i>
Budakeszi	47°30'83"N 18°56'03"E	1962–1970
Erdősmecske	46°10'51"N 18°30'80"E	1970
Felsőtárkány	47°58'44"N 20°25'07"E	1961–1970
Gerla	46°42'01"N 21°11'07"E	1962–1970
Gyulaj	46°30'51"N 18°17'76"E	1970
Makkoshotyka	48°21'52"N 21°31'17"E	1961–1970
Mátraháza	47°46'87"N 19°55'69"E	1961–1970
Répáshuta	48°02'90"N 20°31'70"E	1962–1970
Sopron	47°41'01"N 16°34'79"E	1962–1970
Szakonyfalu	46°55'45"N 16°13'71"E	1967–1970
Szentpéterfőldé	46°37'02"N 16°45'64"E	1968–1970
Szombathely	47°14'01"N 16°37'22"E	1962–1970
Tolna	46°25'60"N 18°46'95"E	1962–1970
Tompa	46°12'28"N 19°38'08"E	1962–1970
Várgesztes	47°28'52"N 18°23'91"E	1962–1970
Zalaerdőd	47°03'44"N 17°03'30"E	1970

For our study, 32 forest phytophagous Macrolepidoptera species were selected from the national forestry light trap network material dating back to the years between 1961 and 1970.

The species were selected based on data available from several light traps over many years.

Table 2. Catching data of caught species

Families and species	Light-traps	Years	Number of		χ^2 P<
			Σ ♂	Σ ♀	
Lasiocampidae					
December Moth <i>Poecilocampa populi</i> (Linnaeus, 1758)	2	9	2,817	317	0.01
Autumn Eggar <i>Eurigaster rimicola</i> (Denis et Schiffermüller, 1775)	1	9	2,027	40	0.01
Barred Hook-tip <i>Watsonalla cultraria</i> (Fabricius, 1775)	3	5	818	1,484	0.01
Scarce Hook-tip <i>Sabra harpagula</i> (Esper, 1786)	2	5	888	134	0.01
Thyatiridae					
Popular Lutestring <i>Tethea or</i> (Denis et Schiffermüller, 1775)	3	8	2,353	929	0.01
Geometridae					
Maiden's Blush <i>Cyclophora punctaria</i> (Linnaeus, 1758)	1	4	1,197	1,331	0.01
Clay Triple-lines <i>Cyclophora linearia</i> (Hübner, 1799)	4	7	4,005	4,864	0.01
November Moth <i>Epirrita dilutata</i> (Denis et Schiffermüller, 1775)	3	2	1,216	114	0.01
Dingy Shell <i>Euchoeca nebulata</i> (Scopoli, 1763)	8	8	2,656	427	0.01
Sharp-angled Peacock <i>Macaria alternata</i> (Denis et Schiffermüller, 1775)	5	9	3,018	1,990	0.01
Featheres Thorn <i>Colotois pennaria</i> (Linnaeus, 1761)	9	7	4,561	567	0.01
Peppered Moth <i>Biston betularia</i> (Linnaeus, 1758)	5	8	4,359	34	0.01
Pale Oak Beauty <i>Hypomecis punctinalis</i> (Scopoli, 1763)	9	9	12,715	2,938	0.01
The Engrailed <i>Ectropis bistortata</i> (Goeze, 1781)	12	9	17,815	1,517	0.01
Notodontidae					
Buff-tip <i>Phalera bucephala</i> (Linnaeus, 1758)	3	4	495	7	0.01
Plumed Prominent <i>Ptilophora plumigera</i> (Denis et Schiffermüller, 1775)	1	4	2,118	275	0.01
Small Chocolate-tip <i>Clostera pigra</i> (Hufnagel, 1766)	1	10	886	12	0.01
Chocolate-tip <i>Clostera curtula</i> (Linnaeus, 1758)	1	10	699	7	0.01

Table 2. Catching data of caught species (continuation)

Families and species	Light-traps	Number of			χ^2 P<
		Years	Σ ♂	Σ ♀	
Lymantriidae					
Pale Tussock <i>Calliteara pudibunda</i> (Linnaeus, 1758)	4	7	1,411	72	0.01
Yellow-tail <i>Euproctis similis</i> (Fuessly, 1775)	2	9	540	38	0.01
White Satin Moth <i>Leucoma salicis</i> (Linnaeus, 1758)	1	5	157	18	0.01
Arctiidae					
Dotted Footman <i>Pelosia muscerda</i> (Hufnagel, 1766)	3	10	1,791	1,762	NS
Scarce Footman <i>Eilema complana</i> (Linnaeus, 1758)	6	10	8,244	6,156	0.01
Common Footman <i>Eilema lurideola</i> (Zincken, 1817)	5	9	15,980	12,252	0.01
Noctuidae					
Small Quaker <i>Orthosia cruda</i> (Denis et Schiffermüller 1775)	5	7	6,820	3,201	0.01
Hebrew Character <i>Orthosia gothica</i> (Linnaeus, 1758)	8	9	4,565	727	0.01
The Satellite <i>Eupsilia transversa</i> (Hufnagel, 1766)	7	10	2,974	2,911	NS
The Chestnut <i>Conistra vaccinii</i> (Linnaeus, 1761)	11	10	11,479	5,428	0.01
Pale-lemon Sallow <i>Xanthia ocellaris</i> (Borkhausen, 1792)	3	8	542	886	0.01
The Dun-bar <i>Cosmia trapezina</i> (Linnaeus, 1758)	6	8	2,097	1,483	0.01
Lesser Belle <i>Colobochyla salicalis</i> (Denis et Schiffermüller, 1775)	2	9	2,056	614	0.01
Jubilee Fan-foot <i>Zancloqnatha lunalis</i> (Scopoli, 1763)	4	10	12,077	8,234	0.01

The flying period and primary food plants of caught moths are shown in Table 3.

3 METHODS

All species were processed separately, but by the same method. The number of captured males and females was counted for the entire swarming. These were summarized and the difference in level of significance was calculated with χ^2 test.

For each swarming we calculated the percentage of males and females. We also calculated the values of the coefficients of variation, which express the deviations in average percentages.

Table 3. The flying period and primary food plants of caught moths

<i>Families and species</i>	<i>Flying period of moths</i>	<i>Primary food plants</i>
Lasiocampidae		
<i>P. populi</i> L.	October-November	Quercus, Betula, Populus, Tilia
<i>E. rimicola</i> Den. et Schiff.	September-October	Quercus
<i>W. cultraria</i> Fabr.	May-June; July-August	Fagus
<i>S. harpagula</i> Esp.	May-June; July-August	Tilia, Betula, Quercus
Thyatiridae		
<i>T. or</i> Den. et Schiff.	April-May; August	Populus
Geometridae		
<i>C. punctaria</i> L.	April-May; July-August	Quercus, Betula
<i>C. linearia</i> Hbn.	May-June; July-August	Fagus, Quercus, Betula
<i>E. dilutata</i> Den. et Schiff.	September-November	Quercus, Acer, Betula, Ulmus
<i>E. nebulata</i> Scop.	June-August	Alnus Quercus, Acer, Betula
<i>M. alternata</i> Den. et Schiff.	April-May; July-August	Salix, Alnus, Prunus
<i>C. pennaria</i> L.	September-November	Carpinus, Quercus, Tilia, Salix
<i>B. betularia</i> L.	May-June; July-August	Betula, Ulmus, Salix, Fraxinus
<i>H. punctinalis</i> Scop.	May-July	Quercus, Betula
<i>E. bistortata</i> Gze.	April-May	Polyphagous (Acer, Alnus)
Notodontidae		
<i>Ph. bucephala</i> L.	May-June ; July-August	Quercus, Tilia, Salix
<i>P. plumigera</i> Den. et Schiff.	October-December	Acer, Fagus, Prunus
<i>C. pigra</i> Hfn.	April-June; July-August	Salix
<i>C. curtula</i> L.	April-May; June-August	Populus, Salix
Lymantriidae		
<i>C. pudibunda</i> L.	May-June	Fagus, Carpinus, Ulmus, Tilia,
<i>E. similis</i> Fuesl.	June-July	Quercus-, Ulmus-, Tilia, Salix
<i>L. salicis</i> L.	June-July	Populus
Arctiidae		
<i>P. muscerda</i> Hfn.	July-August	Lichenes
<i>E. complana</i> L.	July-August	Lichenes
<i>E. lurideola</i> Znck.	July-August	Lichenes
Noctuidae		
<i>O. cruda</i> Den. et Schiff.	March-April	Quercus, Betula, Acer, Carpinus
<i>O. gothica</i> L.	March-April	Quercus, Tilia, Ulmus, Betula
<i>E. transversa</i> Hfn.	September-April	Quercus, Betula
<i>C. vaccinii</i> L.	September-April	Quercus, Betula
<i>X. ocellaris</i> Brkh.	September-April	Quercus, Tilia, Acer, Ulmus,
<i>C. trapezina</i> L.	June-September	Quercus, Fagus, Betula, Acer
<i>C. salicalis</i> Den. et Schiff.	May-August	Populus, Salix
<i>Z. lunalis</i> Scop.	May-September	Fagus

4 RESULTS AND DISCUSSION

The results are shown in Table 4.

Table 4. The percentage of males and females, deviations and coefficients of variation.

Families and species	Σ Moths	Mean σ %	σ s	σ CV	Mean φ %	φ s	φ CV
Lasiocampidae							
<i>Poecilocampa populi</i> L.	2,134	0.83	0.177	0.21	0.17	0.177	1.04
<i>Eriogaster rimicola</i> Den. et Schiff.	2,067	0.98	0.018	0.02	0.02	0.018	0.90
Drepanidae							
<i>Watsonalla cultraria</i> Fabr.	2,308	0.37	0.111	0.30	0.63	0.106	0.17
<i>Sabra harpagula</i> Esp.	1,022	0.88	0.042	0.05	0.12	0.042	0.37
Thyatiridae							
<i>Tethea or</i> Den. et Schiff.	3,292	0.75	0.092	0.12	0.25	0.106	0.42
Geometridae							
<i>Cyclophora punctaria</i> L.	2,528	0.61	0.179	0.29	0.39	0.179	0.46
<i>Cyclophora linearia</i> Hbn.	8,862	0.48	0.102	0.21	0.52	0.092	0.18
<i>Epirrita dilutata</i> Den. et Schiff.	1,340	0.89	0.040	0.04	0.11	0.034	0.31
<i>Euchoeca nebulata</i> Scop.	3,083	0.86	0.141	0.16	0.12	0.141	1.17
<i>Macaria alternata</i> Den. et Schiff.	5,004	0.75	0.114	0.15	0.25	0.115	0.46
<i>Colotois pennaria</i> L.	5,128	0.91	0.086	0.09	0.09	0.086	0.96
<i>Biston betularia</i> L.	4,393	0.99	0.011	0.01	0.01	0.011	1.10
<i>Hypomecis punctinalis</i> Scop.	15,553	0.88	0.076	0.09	0.12	0.076	0.63
<i>Ectropis bistortata</i> Goeze	19,443	0.97	0.048	0.05	0.03	0.045	1.50
Notodontidae							
<i>Phalera bucephala</i> L.	502	0.98	0.019	0.02	0.02	0.019	0.95
<i>Ptilophora plumigera</i> Den. et Schiff.	2,393	0.90	0.054	0.06	0.10	0.054	0.54
<i>Clostera pigra</i> Hfn.	886	0.99	0.013	0.01	0.01	0.008	0.80
<i>Clostera curtula</i> L.	699	0.99	0.009	0.01	0.01	0.009	0.90
Lymantriidae							
<i>Calliteara pudibunda</i> L.	1,183	0.95	0.039	0.04	0.05	0.039	0.78
<i>Euproctis similis</i> Fuesl.	578	0.94	0.037	0.04	0.06	0.036	0.60
<i>Leucoma salicis</i> L.	175	0.89	0.073	0.08	0.11	0.074	0.67
Arctiidae							
<i>Pelosia muscerda</i> Hfn.	3,553	0.53	0.108	0.20	0.47	0.108	0.23
<i>Eilema complana</i> L.	13,400	0.56	0.129	0.23	0.44	0.129	0.29
<i>Eilema lurideola</i> Zinck.	28,232	0.68	0.174	0.26	0.32	0.174	0.54
Noctuidae							
<i>Orthosia cruda</i> Den. et Schiff.	10,021	0.66	0.105	0.16	0.34	0.105	0.31
<i>Orthosia gothica</i> L.	5,292	0.88	0.071	0.08	0.12	0.071	0.59
<i>Eupsilia transversa</i> Hfn.	5,885	0.51	0.083	0.16	0.49	0.083	0.17
<i>Conistra vaccinii</i> L.	16,907	0.69	0.123	0.18	0.31	0.123	0.40
<i>Xanthia ocellaris</i> Borkh.	1,428	0.39	0.194	0.50	0.61	0.194	0.32
<i>Cosmia trapezina</i> L.	3,580	0.58	0.082	0.14	0.42	0.082	0.19
<i>Colobochyla salicalis</i> Denis et Schiff.	2,670	0.81	0.085	0.10	0.19	0.085	0.45
<i>Zanclognatha lunalis</i> Scop.	20,311	0.62	0.130	0.21	0.38	0.130	0.34

Notes: Mean σ % and Mean φ % = averaged percentage of males and females,
 σ s and φ s = deviations, σ CV and φ CV = coefficient of variations.

We found the majority of moths collected in light traps are males. This result is true of 29 species from the investigated 32 species. However, the proportion of males and females of each species, and even within the same species, differed greatly during each swarming.

One probable explanation may be the protandry for greater number of males. Cordillot (1989) established that occurrence of the male of the European corn-borer (*Ostrinia nubilalis* Hbn.) in the light trap preceded the females' occurrence by 3.8 ± 1.5 days on average. This phenomenon was named "protandry" by Stockel and Peyelut (1984). That the females of some species are attracted to light in greater number after mating may be the cause of this.

Yathom (1981) found the most frequently light trapped Egyptian bollworm (*Earias insulana* Boisduval) females mated once. Lopez et al. (2000) found that the overwhelming majority of the females of *Mythimna unipuncta* (Haworth) flew to light after egg-laying. We also observed the "protandry" phenomenon in the case of the dotted footman *Pelosia muscerda* Hfn. in Tompa 1970; the male and female ratio is equal with this species. Males and females of both species (*Pelosia muscerda* Hfn. and *Eupsilia transverse* Hfn.), are captured nearly in similar number. Cordillot (1989) found that the overall ratio of European corn-borer (*Ostrinia nubilalis* Hbn.) was found almost the same; of the captured moths, 52.7% were males and 47.3% were females.

Watsoniana cultraria Fabr. is the only one species captured by light traps that showed a significant female majority. We examined 8 swarmings; females outnumbered males in 7 of them. The males of this moth also fly in the sunlight and they can usually be seen among the higher branches of beech trees. This may be a reason why fewer males of this species are captured at night.

For decades it has been known that the proportion of male and female individuals of various insect species caught by light trap tends to differ. This fact proves that the ratio of the various species represented the catch are not the same as the ratio that appears in nature (Kiss et al. 2003). The reasons for this fact may be many. Flight is difficult for females because of their increased weight due to developing eggs. The males may be more active as they visit the females with the aim of the mating. It is also possible that the males have a greater affinity to light (Waringer 2003).

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Macrofungi in the Botanical Garden of the University of West Hungary, Sopron

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Abstract – Botanical gardens have diverse habitats and floristic conditions. The aim of this study was to examine whether these specific environmental conditions have a positive impact on the appearance of mushrooms. Between 2011 and 2013, mycological observations were performed in the Botanical Garden of the University of West Hungary, Sopron. A total of 171 mushrooms species were identified. Several rare species and two protected species were found. The identification and classification of the species reveal how botanical gardens provide a special habitat for mushrooms. These features of botanical gardens are beneficial for fungal dissemination and preservation.

botanical garden/special habitats/fruit-body monitoring/ex situ conservation

Kivonat – Nagygombák a Nyugat-magyarországi Egyetem Soproni Botanikus Kertjében.

A botanikus kertekben, sajátosságaikból adódóan igen változatos termőhelyi és florisztikai viszonyok vannak. Tanulmányunk célja vizsgálni, hogy ezek a speciális környezeti feltételek milyen kedvező hatással vannak a nagygombák megjelenésére. A 2011-13 években mikológiai megfigyeléseket végeztünk a Nyugat-magyarországi Egyetem soproni botanikus kertjében. Vizsgálataink során összesen 171 nagygomba fajt sikerült kimutatnunk, melyek között számos ritka és két Magyarországon védett faj is előkerült. A fajok meghatározása és csoportosítása rávilágított arra, hogy milyen speciális élőhelyet nyújtanak a botanikus kertek a nagygombáknak. A botanikus kert adottságai lehetőséget nyújtanak a gombák széleskörű megismertetésére és megőrzésre.

botanikus kert/speciális élőhely/termőtest monitoring/ex situ megőrzés

1 INTRODUCTION

The history of European botanical gardens dates back to the Middle Ages. They evolved from early herbalist gardens and were already widespread in the 16th century. Their focus shifted to educational and scientific purposes in the 18th century and reached their present form and role in the 18th-19th centuries (Hill 1915). The main objectives of botanical gardens today are to collect, preserve, and exhibit plants native to a given location as well as more colorful or rare non-native species (Brickell 1991, Dongyan – Zuoshuang 2008). These types of gardens have become increasingly important today. Their artificial habitats, diverse flora, and habitat conditions are favorable for mushroom species as well.

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The aim of this study is to illustrate the important role such gardens (e.g. arboretums, botanical gardens, parks) play not only in the preservation of flora, but also in the equally important preservation of mushrooms. We demonstrate this with the example of the Botanical Garden of the University of West Hungary in Sopron.

There are several studies that examine mushrooms occurring in urban environments. Most of these studies focus on the many rare and protected species that appear in urban environments and explore how these urban environments are especially favorable for parasitic and saprotrophic species (Luszczynski 1997, Pál-Fám 2001, Pál-Fám – Boros 2006). The mycological importance of urban parks and gardens emphasizes the differences that exist among artificially created environments and disturbed environments. These green urban areas are an important habitat for mushrooms (Lisiewska – Strakulska 2002, Kasprovicz et al 2011, Skorupski et al 2011, Bujakiewicz – Kujawa 2000). For example, during a five-year study of the People's Garden of Miskolc, a total of 124 species were identified. Of these, only 33 species were mycorrhizal, but several rare and protected species were also found (Kaposvári 2013). Fungi observation in the botanical garden of Soroksár began 40 years ago (Konecsni et al. 1973); since then 274 fungi species have been identified, including a number of rare and protected species (Rimóczy 1993, 1998). A list of 58 species of macromycetes was recorded in the Warsaw Botanical Garden between 1961 and 1963 and is discussed in a paper by Szober (1965). The Central Botanical Garden of Belarus in Minsk recorded 12 species of macromycetes, which is the topic of a paper by Dischuk (2001). In one of his studies, Szczepkowski (2007) identified 79 species of macromycetes in the Dendrological Park of the Warsaw Agricultural University in Warsaw between 1996 and 2005. Arboretums also favor the diversity of the lichen fungi species (Ladd et al 2009). A more preferred topic within the study of fungi in urban environments is the examination of mushrooms nurtured in glasshouses and greenhouses where tropical species can often be found (Pidlich-Aigner et al. 2002, Gubitz 2012, Lukács et al. 2010, 2011). Szczepkowski et al. (2014) reported a total of 206 species that existed in greenhouses in five large European cities. Arboretums also favor the diversity of lichen fungi species (Ladd et al. 2009).

2 MATERIALS AND METHODS

2.1 Description of the study area

The Botanical Garden of the University of West Hungary is located in Sopron near the north-western border of Hungary and Austria. Geographically it lies in the eastern foothills of the Sopron hills. From a geobotanical point of view, it is considered a border of the floristic region of the Eastern Alps (Alpicum, subdivision "Ceticum") and the Pannonian basin (Pannonicum, subdivision "Castriferreicum"). Afforestation of the area the Botanical Garden of Sopron occupies today began in 1897; before that, it was home to oak forests, orchards, and kitchen gardens. The development of the Botanical Garden began after 1923 and evolved on 17.2 hectares; the area was primarily used for teaching and practicing forestry. Later, new plantations in plant-geographical clusters were created. Thus, groups of East Asian and North American woody flora evolved (Nemky – Vancsura 1970, Kocsó 1996, 2008). Typically the garden contains a variety of temperate zone plants in its collection and international seed exchanges are largely responsible for their expansion (Kocsó, 1996). Two of the most important missions of the Botanical Garden are to collect a great many and a great variety of taxa, and also to conserve many species (also protected and not threatened) in situ and ex situ. In 2007 the garden contained 2691 woody plant taxa, and 3691 herbaceous taxa and, due to the varied plant cover, there were 57 moss species as well (Kocsó 2008, Szücs 2008). Among the plant taxa were 16 in situ protected species and 135 ex situ protected species (Kocsó 2008).

The soils of the Botanical Garden were developed on loess, marl and marl-like sediments, and they belong to the reference soil groups of Cambisols and Luvisols. The soil texture is loam, clayey-loam, and the structure is well-formed subangular and angular blocky. The nutrient and water supply statuses of the upper soil horizons are good for weak acidic soil pH. Rain water flows here from the eastern foothills of the Sopron hills providing the area with good water conditions. Due to its geographical location, the garden has a sub-Alpine climate. The average annual rainfall is 690 mm; the 12-hour humidity in July is about 56%, which is a good climate for hornbeam and oak (Cserpes – Kocsó 1996).

2.2 Macrofungi investigation

The mycological observations in the Botanical Garden began in 2004, but our field surveys were completed between 2011 and 2013. The number of surveys varied from six to eight times per year and were weather dependent: there were 1-2 surveys in the spring and 3-4 in the summer and autumn. As the university is located in the garden, informal observations were made frequently.

Table 1.: Date of macrofungi observation

Years	Spring	Summer	Autumn
2011	22.05.	05.06.;	25.09.; 04.10., 21.10.; 07.11.
2012	25.05.	05.06.; 14.06.; 17.07.; 24-27.07.;	20.09.; 28.09.; 01-05.10.; 17-19.10.; 24.10.; 08.11.
2013	07.05.; 17.05.;	05-07.06.; 25.06.; 26.08.;	05-06.09.; 01-12.09.; 18-19.09.; 01-03.10.; 08-10.10.; 16-18.10.

Observations of the mushrooms were regularly carried out by exploring the entire Botanical Garden utilizing the garden paths after major rainfalls (*Figure 1.*). The Botanical Garden has an extremely diverse flora which made it impossible to prepare individual sampling areas.

During the surveys, the number of locations where fruiting bodies of different species were found was recorded (*Table 2.*). If this occurred in the same location over several years, it is not calculated as a different position. The mushrooms were identified in the field, photo documented and, in some cases, fungarium were also completed. The species were identified by their macroscopical and ecological specifics, as well as their microscopical specifics (cystidium and spore measurements). The spore and cystidia observations were made using a Nikon H600L and a Zeiss Axio Imager light microscope at 600× and 1000× magnification. Images were analyzed with Image Pro Plus 7.0 software. If needed, chemicals reagents were used to identify the fungus (e.g. 10% KOH, Meltzer-reagent, or by the cystidium observation kongo-red dye stuff was used). The following literature was used during the identification: Kundes – Vesterholt (2012), Bohus et al. (1951), Igmándy (1991), Galli (1996), Krigelsteiner (2000 a, b.), Aronsen (2012), Assoyov – Miksik (2013), Froslev – Stjernegaard (2013), Tulloss – Yang (2014), Bandini (2014).

Nomenclature followed the Mycobank method (Robert et al. 2014). The species found are listed in *Table 2* with the name of the taxon and the frequency index (rA = relative abundance, from 1 to 5; 1 = very rare, 5 = very common) [how frequently the species was observed during survey time, how abundantly it was found], number of location (Lo). In summary, the Lo value points to a spatial frequency, and the value of rA is the temporal frequency of the appearances.



Figure 1: Map of the botanical garden paths used during the observations

The species found were classified on the basis of Arnolds et al. (1995) and our observations (Ls; m = mycorrhizal, p = parasite (also necrotorf and biotrof), sl = saprotrophic lignicole, st = saprotrophic terricole, sc = saprotrophic on crop residues). In questionable cases (e.g. *Clitopilus prunulus*) the work of Rinaldi et al. (2008) was consulted. A conservation assessment was also made based on the works of Rimóczi et al. (1999) and Siller et al. (2005, 2006), taking into account the changes in Hungarian law (Magyar Közlöny 2013). The conservation assessment is in the rightmost column (P) (0 = Extinct 1 = Critically Endangered; 2 = Endangered; 3 = Vulnerable; 4 = Lower Risk; (Rimóczi et al. 1999). The two species highlighted with bold letters are protected by law in Hungary (Magyar Közlöny 2013)

3 RESULTS, CONCLUSIONS

Previously Kocsó (2008) mentioned the existence of 75 species in the Botanical Garden, but he does not name them. In the period between 2011 and 2013, 171 mushroom taxa were identified in the Botanical Garden of the University of West Hungary, Sopron (see Table 2 in Appendix).

Categorized by life form, 33% of the species are mycorrhizal, 5% are parasitcal, 22% are saprotrophic lignicole, and 40% are saprotrophic terricole or grow on plant debris. The overall species composition does not differ notably from the composition found in the Sopron hills (Folcz et al. 2013). Among the species are calciphilous and acidophilous species as well.

When the set of species is compared with data from the Sopron region, we see that the identified species partially overlap in the Dudlesz forest (which has more calcareous substrates) (Frank 1997) and also with the fungi in the Sopron mountains (which is more acidic) (Folcz et al. 2013). In other words, a sorting of blending area exists between the two mushroom worlds which is in line with the growing conditions of the landscape site. Among the species, there are a total of 15 that are yet unpublished for the Sopron area: *Amanita lividopallescens* (Sacc. ex Boud.) Kühner & Romagn., *Bolbitius titubans* (Bull.) Fr., *Clitocybe costata* Kühner & Romagn., *Galerina laevis* Singer, *Hygrocybe subglobispora* f. *aurantiorubra* Arnolds, *Hygrophorus mesotephrus* Berk. & Broome, *Hohenbuehelia petaloides* (Bull.) Schulzer, *Hemimycena cucullata* (Pers.) Singer s.l., *Lactarius sanguifluus* (Paulet) Fr., *Leucoagaricus americanus* (Peck) Vellinga, *Macrotyphula fistulosa* (Holmsk.) R.H. Petersen, *Melanogaster variegatus* (Vittad.) Tul. & C. Tul., *Pluteus ephebeus* (Fr.) Gillet, *Stropharia coronilla* (Bull. ex DC.) Qué., *Tephrocybe rancida* (Fr.) Donk. Two protected: *Agaricus bohusii* Bon, *Polyporus tuberaster* (Jacq. ex Pers.) Fr, ten endangered species, and many rare species occur among the species. Of the species, 42% (71 species) are on the recommended red list of Hungarian fungi, thus the Botanical Garden plays a significant role in the protection of fungi.

4 DISCUSSION

The diversity of vegetation and the special care and treatment inherent to botanical gardens help create exceptional conditions which are highly favorable to the colonization of fungi. The artificial and disturbed soil conditions are favorable for a variety of terrestrial fungi and the diverse flora have a positive effect on mycorrhizal colonization. The garden is cultivated in such a manner that dead wood and wood debris are allowed to accumulate on the forest floor, so the rate of the lignicole mushrooms is similar to that found in natural forests which are also rich in dead wood. Many of the species appear regularly, others appear rarely, which can be traced back to meteorological conditions during the period. Hawksworth (1991) called attention the importance of international protection of fungi. He underlined the importance of both ex-situ and in-situ methods. Moore et al. (2001) recently highlighted the importance of in situ fungi conservation which stresses habitat protection as a key step in fungi protection. The safeguarding of mushrooms in Italy can also be highlighted as an example. One of the cornerstones of this habitat preservation (Venturelli et al. 2011), is the defense of the arbuscular mycorrhizal fungi (Turrini – Giovanetti 2012). In the case of botanical gardens, exsitu mushroom protection is also possible because the gardens are artificial habitats. The rare and protected species that were found show that botanical gardens may be suitable for this task of habitat support if we keep in mind the ex situ (or sometimes the in situ) conservation of the forming and sustaining fungi. Based on our results, botanical gardens are very important habitats for fungi and this provides many opportunities both for science and for active fungi protection (Szober 1965, Pál-Fám et al 2004, Szczepowski 2007). The (ex situ) conservation in such places creates opportunities for mycological research and educational activities and for mycological biodiversity preservation (Hu – Zhang 2008, Varese et al. 2011).

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APPENDIX

Table 2. The observed macrofungi species in the botanical garden of the University of West Hungary, Sopron

Nr.	Species	rA	Lo	Ls	P
Ascomycota					
1	<i>Ascocoryne sarcoides</i> (Jacq.) J.W. Groves & D.E.	1	1	sl	3
3	<i>Helvella crispa</i> (Scop.) Fr.	4	6	st	3
4	<i>Helvella elastica</i> Bull.	1	2	st	3
5	<i>Humaria hemisphaerica</i> (F.H. Wigg.) Fuckel	1	2	st	4
6	<i>Kretzschmaria deusta</i> (Hoffm.) P.M.D. Martin	1	1	p	–
7	<i>Tarzetta cupularis</i> (L.) Svrček	1	1	st	4
8	<i>Morchella esculenta</i> (L.) Pers.	1	1	st	3
9	<i>Peziza varia</i> (Hedw.) Alb. & Schwein.	3	2	sl	4
10	<i>Sarcoscypha austriaca</i> (Beck ex Sacc.) Boud.	2	3	sl	4
11	<i>Xylaria hypoxylon</i> (L.) Grev.	1	2	sl	–
12	<i>Xylaria polymorpha</i> (Pers.) Grev	1	3	sl	–
Bazidiomycota					
13	<i>Agaricus augustus</i> Fr.	1	2	st	2
14	<i>Agaricus bitorquis</i> (Quél.) Sacc.	2	1	st	–
15	<i>Agaricus bohusii</i> Bon	1	1	st	2
16	<i>Agaricus campestris</i> L.	1	1	st	–
17	<i>Agaricus sylvaticus</i> Schaeff.	2	3	st	–
18	<i>Agaricus xanthodermus</i> Genev.	5	10	st	–
19	<i>Agrocybe erebia</i> (Fr.) Kühn.	1	1	st	2
20	<i>Agrocybe praecox</i> (Pers.) Fayod	2	2	st	–
21	<i>Amanita battarrae</i> (Boud.) Bon	1	1	m	3
22	<i>Amanita citrina</i> (Schaeff.) Pers.	1	1	m	3
23	<i>Amanita lividopallescens</i> (Secr. ex Boud.) Kühner & Romagn.	2	1	m	2
24	<i>Amanita muscaria</i> (L.) Lam.	2	2	m	3
25	<i>Amanita phalloides</i> var. <i>phalloides</i> (Vaill. ex Fr.) Link	3	3	m	–
26	<i>Amanita rubescens</i> Pers.	1	1	m	–
27	<i>Amanita solitaria</i> (Bull.) Mérat	2	3	m	2
28	<i>Amanita strobiliformis</i> (Paul.: Vitt.) Bertil.	2	4	m	3
29	<i>Armillaria mellea</i> (Vahl.) P. Kumm.	1	1	p	–
30	<i>Armillaria ostoyae</i> (Romagn.) Herink	1	2	p	–
31	<i>Armillaria tabescens</i> (Vahl.) P. Kumm.	2	1	p	–
32	<i>Auricularia auricula-judae</i> (Bull.) Quél.	1	1	sl	–
33	<i>Auriscalpium vulgare</i> Gray	3	2	sl	–
34	<i>Bolbitius titubans</i> (Bull.) Fr.	3	5	sc	3
35	<i>Boletus impolitus</i> Fr.	1	1	m	4
36	<i>Boletus luridus</i> Sowerby	2	3	m	4
37	<i>Boletus radicans</i> Pers.	3	2	m	3
38	<i>Calocybe gambosa</i> (Fr.) Donk	1	1	st	–
39	<i>Chlorophyllum olivieri</i> (Barla) Vellinga	3	7	st	–
40	<i>Chroogomphus rutilus</i> (Schaeff.) O.K. Mill.	2	2	m	–
41	<i>Clitocybe costata</i> Kühner & Romagn	1	1	st	3
42	<i>Clitocybe gibba</i> (Pers.) P. Kumm.	2	2	st	3

Nr.	Species	rA	Lo	Ls	P
43	<i>Clitocybe nebularis</i> (Batsch) P. Kumm.	1	2	st	3
44	<i>Clitocybe odora</i> (Bull.) P. Kumm.	1	1	st	3
45	<i>Clitocybe rivulosa</i> (Pers.) P. Kumm.	1	2	st	3
46	<i>Clitocybula platyphylla</i> (Pers.) Malençon & Bertault	1	2	st	–
47	<i>Clitopilus prunulus</i> (Scop.) P. Kumm.	2	4	st	–
48	<i>Coprinellus disseminatus</i> (Pers.) J. E. Lange	4	7	sl	–
49	<i>Coprinellus micaceus</i> (Bull.) Vilgalys, Hopple & Jacq. Johnson	5	6	sl	–
50	<i>Coprinopsis atramentaria</i> (Bull.) Redhead, Vilgalys & Moncalvo	1	1	sl	–
51	<i>Coprinus comatus</i> (O. F. Müll.) Pers.	4	5	st	–
52	<i>Coprinus leiocephalus</i> P.D. Orton	1	1	st	–
53	<i>Coprinus cinereus</i> S.F. Gray	1	1	sc	–
54	<i>Cortinarius infractus</i> Berk. s. l.	1	1	m	–
55	<i>Cortinarius largus</i> Fr.	1	1	m	3
56	<i>Cortinarius subpurpurascens</i> (Batsch) Fr.	1	2	m	3
57	<i>Cortinarius trivialis</i> J. E. Lange s. l.	2	1	m	–
58	<i>Crepidotus mollis</i> (Schaeff.) Staudé	1	1	sl	–
59	<i>Cyathus olla</i> (Batsch) Pers.	2	3	sl	–
60	<i>Cyathus striatus</i> (Huds.) Willd.	3	4	sl	–
61	<i>Dacrymyces chrysospermus</i> Berk. & M.A. Curtis	1	2	sl	–
62	<i>Daedalea quercina</i> (L.) Pers.	1	1	sl	–
63	<i>Entoloma rhodopolium</i> (Fr.) P. Kumm.	1	1	m	–
64	<i>Exidia nigricans</i> (With.) P. Roberts	1	2	sl	–
65	<i>Flammulina velutipes</i> (Curtis) Singer	1	2	p	–
66	<i>Fomitopsis pinicola</i> (Sw.) P. Karst	1	1	p	–
67	<i>Galerina laevis</i> (Pers.) Singer	1	3	st	3
68	<i>Geastrum fimbriatum</i> Fr.	1	1	st	3
69	<i>Geastrum triplex</i> Jungh.	3	5	st	3
70	<i>Gomphidius glutinosus</i> (Schaeff.) Fr.	2	2	m	–
71	<i>Gymnopus dryophilus</i> (Bull.) Murrill	5	9	st	–
72	<i>Gymnopus fusipes</i> (Bull.) Gray	1	1	sl	–
73	<i>Hebeloma crustuliniforme</i> (Bull.) Quéf.	1	2	m	–
74	<i>Hemimycena cucullata</i> (Pers.) Singer s. l.	2	3	st	2
75	<i>Hemiphiliota populnea</i> (Pers.) Bon	1	1	sl	–
76	<i>Hohenbuehelia petaloides</i> (Bull.) Schulzer	1	1	st	3
77	<i>Hygrocybe ceracea</i> (Wulf.) P. Kummer.	1	1	st	3
78	<i>Hygrocybe subglobispora</i> f. <i>aurantiorubra</i> Arnolds	1	1	st	2
79	<i>Hygrocybe virginea</i> (Wulfen) P.D. Orton & Watling	1	1	st	2
80	<i>Hygrophoropsis aurantiaca</i> (Wulfen) Maire	1	2	st	–
81	<i>Hygrophorus hypothejus</i> (Fr.:Fr.)Fr.	1	1	m	3
82	<i>Hygrophorus mesotephros</i> Berk. & Broome	1	1	m	3
83	<i>Hypholoma fasciculare</i> (Huds.) P. Kumm.	2	5	sl	–
84	<i>Hypholoma lateritium</i> (Schaeff.) P. Kumm.	1	1	sl	–
85	<i>Infundibulicybe geotropa</i> (Bull.) Harmaja	1	1	st	–
86	<i>Inocybe asterospora</i> Quéf. s. l.	1	1	m	3
87	<i>Inocybe flocculosa</i> Sacc. s. l.	1	1	m	3
88	<i>Inocybe geophylla</i> (Bull.) P. Kumm.	1	3	m	3
89	<i>Inocybe rimosa</i> (Bull.) P. Kumm.	1	3	m	–
90	<i>Laccaria amethystina</i> Cooke	1	1	m	3

Nr.	Species	rA	Lo	Ls	P
91	<i>Laccaria bicolor</i> (Maire) P.D. Orton	1	1	m	3
92	<i>Lacrymaria lacrymabunda</i> (Bull.) Pat.	4	7	st	–
93	<i>Lactarius aurantiacus</i> (Pers.) Gray	1	2	m	–
94	<i>Lactarius blennius</i> (Fr.) Fr.	1	1	m	–
95	<i>Lactarius chrysorrheus</i> Fr.	1	2	m	–
96	<i>Lactarius circellatus</i> Fr.	1	1	m	–
97	<i>Lactarius decipiens</i> Quél.	1	1	m	–
98	<i>Lactarius deliciosus</i> (L.) Gray	1	1	m	4
99	<i>Lactarius deterrimus</i> Gröger	1	2	m	–
100	<i>Lactarius quietus</i> (Fr.) Fr.	3	7	m	–
101	<i>Lactarius sanguifluus</i> (Paulet) Fr.	2	1	m	–
102	<i>Lactarius torminosus</i> (Schaeff.) Gray	1	1	m	4
103	<i>Lactarius turpis</i> (Weinm.) Fr.	1	2	m	3
104	<i>Leccinum pseudoscabrum</i> (Kallenb.) Šutara	1	1	m	4
105	<i>Lentinus tigrinus</i> (Bull.) Fr.	1	1	sl	–
106	<i>Lepiota cristata</i> P. Kumm.	2	6	st	–
107	<i>Lepista inversa</i> (Scop.) Pat.	4	5	st	–
108	<i>Lepista luscina</i> (Fr.) Singer	1	1	st	–
109	<i>Lepista nuda</i> (Bull.) Cooke	2	5	st	–
110	<i>Lepista saeva</i> (Fr.) P. D. Orton	1	2	st	–
111	<i>Lepista sordida</i> (Schumach.) Singer	1	2	st	–
112	<i>Leucoagaricus americanus</i> (Peck) Vellinga	1	1	st	2
113	<i>Leucoagaricus leucothites</i> (Vittad.) Wasser	2	3	st	–
114	<i>Lycoperdon perlatum</i> Pers.	1	1	st	–
115	<i>Lycoperdon pratense</i> Pers.	1	1	st	2
116	<i>Lyophyllum decastes</i> (Fr.) Singer	2	4	st	–
117	<i>Macrolepiota mastoidea</i> (Fr.) Singer	1	1	st	–
118	<i>Macrotyphula fistulosa</i> (Holmsk.) R.H. Petersen	1	1	sl	3
119	<i>Marasmius oreades</i> (Bolton) Fr.	1	1	sc	–
120	<i>Melanogaster variegatus</i> (Vittad.) Tul. & C. Tul.	1	2	m	3
121	<i>Melanoleuca melaleuca</i> (Pers.) Murrill	1	1	st	–
122	<i>Mycena galericulata</i> (Scop.) Gray	1	1	st	–
123	<i>Mycena polygramma</i> (Bull.) Gray	1	1	sl	–
124	<i>Mycena pura</i> (Pers.) P. Kumm.	3	7	st	–
125	<i>Mycena rosea</i> Gramberg	1	2	st	–
126	<i>Panellus stipticus</i> (Bull.) P. Karst.	1	1	sl	–
127	<i>Paxillus involutus</i> (Batsch) Fr.	1	2	m	–
128	<i>Pholiota squarrosa</i> (Vahl) P. Kumm.	1	1	sl	3
129	<i>Placodes betulinus</i> (Bull.) Quél.	1	2	p	–
130	<i>Pleurotus ostreatus</i> (Jacq.) P. Kumm.	1	2	p	–
131	<i>Pluteus atromarginatus</i> (Konrad) Kühner	1	1	sl	4
132	<i>Pluteus cervinus</i> (Schaeff.) P. Kumm.	3	8	sl	–
133	<i>Pluteus ephebeus</i> (Fr.) Gillet	1	1	sl	4
134	<i>Polyporus arcularius</i> (Batsch) Fr.	1	1	sl	–
135	<i>Polyporus squamosus</i> (Huds.) Fr.	1	2	p	–
136	<i>Polyporus tuberaster</i> (Jacq. ex Pers.) Fr	1	1	sl	3
137	<i>Psathyrella artemisiae</i> (Pass.) Konrad & Maubl. s. l.	1	1	sl	3
138	<i>Psathyrella candolleana</i> (Fr.) Maire	1	1	sl	–

Nr.	Species	rA	Lo	Ls	P
139	<i>Psathyrella marcescibilis</i> (Britzelm.) Singer	3	8	st	3
140	<i>Psathyrella multipedata</i> (Peck) A. H. Sm.	1	1	sl	3
141	<i>Psathyrella piluliformis</i> (Bull.) P. D. Orton	1	2	sl	–
142	<i>Ramaria stricta</i> (Pers.) Quél.	1	2	sl	–
143	<i>Rhodocollybia butyracea</i> (Bull.) Lennox	3	6	st	–
144	<i>Rhodocybe gemina</i> (Paulet) Kuyper & Noordel.	1	2	st	3
145	<i>Russula cyanoxantha</i> (Schaeff.) Fr.	1	2	m	–
146	<i>Russula delica</i> Fr.	1	1	m	–
147	<i>Russula emetica</i> (Schaeff.) Pers. s. l.	1	1	m	3
148	<i>Russula foetens</i> Pers.	2	4	m	–
149	<i>Russula lepida</i> Fr.	1	1	m	3
150	<i>Russula undulata</i> Velen.	1	2	m	3
151	<i>Schizophyllum commune</i> Fr.	4	12	sl	–
152	<i>Scleroderma citrinum</i> Pers.	2	4	m	4
153	<i>Scleroderma verrucosum</i> (Bull.) Pers.	2	3	m	4
154	<i>Stereum hirsutum</i> (Willd.) Pers.	1	2	sl	–
155	<i>Strobilurus stephanocystis</i> (Kühner & Romagn. ex Hora) Singer	1	1	sc	–
156	<i>Stropharia aeruginosa</i> (Curtis) Quél.	3	6	st	–
157	<i>Stropharia coronilla</i> (Bull. ex DC.) Quél.	1	2	st	–
158	<i>Stropharia rugosoannulata</i> Farl. ex Murrill	1	1	st	3
159	<i>Suillus granulatus</i> (L.) Roussel	5	15	m	–
160	<i>Suillus grevillei</i> (Klotzsch) Singer	2	4	m	–
161	<i>Suillus viscidus</i> (L.) Roussel	2	6	m	3
162	<i>Tephrocybe rancida</i> (Fr.) Donk	1	1	st	3
163	<i>Trametes versicolor</i> (L.) Lloyd	2	3	sl	–
164	<i>Tricholoma batschii</i> Gulden	1	4	m	3
165	<i>Tricholoma scalpturatum</i> (Fr.) Quél.	2	2	m	3
166	<i>Tricholoma terreum</i> (Schaeff.) P. Kumm.	3	5	m	–
167	<i>Tubaria furfuracea</i> (Pers.) Gillet	1	2	sl	–
168	<i>Volvopluteus gloiocephalus</i> (DC.) Vizzini, Contu & Justo	1	3	sl	–
169	<i>Xerocomus cisalpinus</i> Simonini, H. Ladurner & Peintner	1	1	m	4
170	<i>Xerocomus porosporus</i> (Imler ex Bon & G. Moreno) Contu	1	1	m	4
171	<i>Xerula radicata</i> (Relhan) Dörfelt.	4	10	sl	–

On the Occurrence of Natural Norway Spruce Woodland in the Pieniny Mts (Western Carpathians)

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Abstract – According to Kulczyński (1928), a natural *Picea abies* forest occurs in the mountain range of the Pieniny Mts on the slopes of the mountain Vysoké skalky. Later, various data on the altitudinal range of this stand (and thus on the locality itself) were published: (1) 890 (900) – 950 m, (2) 1 000 – 1 050 m or (3) around 1 000 m. The aim of the field research was to verify published data on the occurrence of an Upper Mountain Norway spruce woodland (association *Polysticho-Piceetum*) in the territory of Vysoké skalky. A field study in the area has revealed that most of published data on the occurrence of the Kulczyński's *Picea* woodland are incorrect, as its real upper altitudinal limit reaches $\pm$ 940 m. The main result is the confirmation of the real existence of a *Vaccinium myrtillus*-*Homogyne alpina*-*Picea abies* phytocoenosis in the territory of Vysoké skalky. However, tree species other than *Picea abies* (e.g. *Fagus sylvatica*, *Abies alba*, *Acer pseudoplatanus*) could persist in the special habitat of Kulczyński's 'Picea woodland'. The natural vegetation of this place was formed by mixed forest stand.

Malé Pieniny Mts / *Picea abies* woodland / phytosociology / *Polysticho-Piceetum* / Vysoké skalky

Kivonat – A közönséges luc természetes állománya a Pieninekben (Nyugati-Kárpátok). Kulczyński (1928) természetes lucos állomány előfordulását jelezte a Pieninek montán régiójában (a Vysoké skalky csúcs térségében). A későbbiekben ezen állomány tengerszintfeletti magasságáról (s egyáltalán elhelyezkedéséről) különböző adatokat közöltek: (1) 890 (900)–950 m, (2) 1 000–1 050 m vagy (3) 1 000 m körül. A terepi kutatások célja a montán lucfenyves társulás (*Polysticho-Piceetum*) előfordulásának felülvizsgálata volt a Vysoké skalky térségében. A vizsgálatok rávilágítottak arra, hogy a Kulczyński-féle állományra vonatkozó adatok többsége téves. A felmérés megerősítette egy *Vaccinium myrtillus*-*Homogyne alpina*-*Picea abies* növényközösség előfordulását a területen (ennek felső elterjedési határa 940 m tszf. m. körül található), azonban súlypontos más lombos fafajok (pl. *Fagus sylvatica*, *Abies alba*, *Acer pseudoplatanus*) előfordulása is a térségben, azaz a természetes erdőtársulás fetételezhetően egy montán, lombelegyes fenyves lehetett.

Kis-Pieninek / *Picea abies* állományok / növénytársulástan / *Polysticho-Piceetum* / Vysoké skalky

1 INTRODUCTION

A distinct feature of the landscape of higher mountain ranges of the Western Carpathians is formation of a separate altitudinal vegetational zone dominated by native *Picea abies* forests (class *Piceetea excelsae* Klika 1948). According to the different ecological conditions and corresponding floristical variance, several associations were described that are divided into

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two major groups (Kučera 2012a): (A) *Piceetalia excelsae* Pawłowski ex Pawłowski et al. 1928 on generally nutrient-poor acidic substrates and (B) *Athyrio filicis-feminae-Piceetalia* Hadač ex Hadač et al. 1969 on calcareous substrates. In the Slovak part of the Western Carpathians, seven associations among the two major groups of *Picea abies*-dominated plant communities are currently recognised in the present (Kučera 2012a): (A) *Vaccinio myrtilli-Piceetum*, *Athyrio alpestris-Piceetum*, and *Chrysanthemo rotundifolii-Piceetum* and (B) *Seslerio variaie-Piceetum*, *Corthuso matthioli-Piceetum*, *Adenostylo alliariae-Piceetum excelsae*, and *Oxalido acetosellae-Piceetum*. In the Polish phytocoenological literature there are two traditionally differentiated units of supramontane *Picea abies*-dominated communities (see J. M. Matuszkiewicz 2002): *Plagiothecio-Piceetum tatricum* (name is validly published only with the epithet 'tatricum'; a syntaxonomic synonym to *Vaccinio myrtilli-Piceetum* Šoltés 1976) and *Polysticho-Piceetum* (a syntaxonomic (heterotypical) synonym to *Oxalido acetosellae-Piceetum* Březina et Hadač in Hadač et al. 1969 (Kučera 2012a)).

The first evidence of the existence of a native Norway spruce stand (*Picea abies*) in the Pieniny Mts was published by Kulczyński (1928) in the famous work *The plant associations of the Pieniny*. This author gave a short introduction to the differentiation of Carpathians' forests into altitudinal vegetation zones and pointed out that the typical form of *Picea abies* forests could also exist out of the elevation range 1 200–1 550 m (i.e. altitudinal range usually considered as a territory of native supramontane *Picea abies* forests) as a result of natural conditions.

In the Pieniny Mts, reaching up to only 1 049.8 m (Vysoké skalky/Wysokie Skalky/Wysoka in the eastern part of the Pieniny Mts – the so-called Malé Pieniny/Male Pieniny), he found an example of such 'extrazonal' occurrence, which he regarded as a natural phytocoenosis:

"Auf dem Gebiete des Pieniny-Zuges tritt der natürliche Fichtenwald nur an einer einzigen Stelle, unterhalb des Gipfels »Wysokie Skalki«, in einer Höhe von ca. 900 ü. M. auf. Er ist als ein kleines Assoziationsindividuum von einigen Hektaren Oberfläche ausgebildet." (Kulczyński 1928, p. 119).

A very brief description and evaluation of this stand situated at the Polish slopes of Vysoké skalky is followed by a complete listing of species found in the whole stand, with cover-abundance values (Kulczyński 1928, p. 119–120).

To date, I have not found published phytosociological relevés from the area of Kulczyński's *Picea* stand of the Malé Pieniny in the available literature sources. Later authors have only mentioned occurrence of a special vegetation type of *Picea abies* in the Pieniny Mts, mostly without reference to Kulczyński (1928). J. Matuszkiewicz (1977) evaluated Kulczyński's stand within the association *Polysticho lonchitidis-Piceetum* W. Matuszkiewicz ex J. Matuszkiewicz 1977 and utilized the data of Kulczyński (1928) as a 'phytosociological relevé' (see Results).

Reference to the occurrence of the association *Polysticho-Piceetum* in the Pieniny Mts was repeated by W. Matuszkiewicz (1982, 2014), W. Matuszkiewicz – J. M. Matuszkiewicz (1996) and J. M. Matuszkiewicz (2002). In contrast, Medwecka-Kornaś (1959, 1972) did not include the Pieniny Mts in the list of mountain ranges with occurrence of a vegetation type of Norway spruce in her overview of woodland and scrub communities of Poland.

Localization of the occurrence of the *Picea abies* stand under consideration (Malé Pieniny, on the slope of Vysoké skalky) varies in the publications. According to the first author, Kulczyński (1928), his hectares of *Piceetum excelsae* occur in the elevation ca. 900 m. In the chapter of Zarzycki (1982, Tab. I), the altitudinal range of *Piceetum excelsae* occurrence is limited to elevations of 890–950 m. The same values are given in Tab. I by Zarzycki (1981, p. 14), although in the text (p. 32) the indicated values are 900–950 m.

A somewhat different place is given by W. Matuszkiewicz (1982, p. 216–217, *Polysticho-Piceetum*; 2014): in the elevation ca. 1 000–1 050 m below the summit of

Wysokie Skalki. This location was apparently followed by Szeląg (1995) as he wrote that *Polysticho-Piceetum* occurs only in the summit part of the Wysokie Skalki, at the elevation 1 000–1 050 m. In the same book as Zarzycki (see above), Pancer-Kotejowa et al. (1982, p. 319) mentioned an elevation ‘around 1 000 m’.

In the third group of works, there is no exact elevation range in metres given, e.g. J. Matuszkiewicz (1977): on the highest summit of the Malé Pieniny; Pancer-Kotejowa et al. (1982) on page 325 did not give exact data on location (see above).

In respect to the overall distribution of natural non-mixed *Picea abies* forests in the Western Carpathians (Matuszkiewicz 2002, Kučera 2012a, cf. Kulczyński 1928, p. 118), published data on the occurrence of a natural *Picea abies* community in the Pieniny Mts are very interesting. Such a unique existence was probably one of the reasons why this area is under protection in the form of a nature reserve, declared several decades ago. As the original data was published only by Kulczyński (1928), I was drawn to understand the nature of such special *Piceetum* occurrence to supplement my knowledge of Western Carpathians’ *Picea abies* woodlands and to gain field experience and data by myself. Verification of published data on the occurrence of a natural Norway spruce woodland in the territory of Vysoké skalky was the aim of the undertaken research: its results are presented in this paper.

2 MATERIAL AND METHODS

Phytosociological relevés were carried out in the year 2011 in cover-abundance scale in terms of Braun-Blanquet (1951) and extended with degrees 2a and 2b according to Barkman et al. (1964) (cf. Westhoff – van der Maarel 1973). Examples of the recorded phytocoenoses (7 phytosociological relevés) are given in the Appendix. Coordinates (WGS-84) were obtained with the device GPSMAP® 60CSx in addition to a hiking map of Pieninský národný park (2001). The age and height of trees was estimated (cf. notes in Kučera 2013). Nomenclature of the vascular plants and bryophytes follows the lists of Marhold et al. (1998) and Kubinská – Janovicová (1998). Collected specimens of bryophytes (herbarium BBZ) were determined by Mgr A. Petrášová and chosen vascular plants by RNDr D. Bernátová, CSc. The programs Turboveg for Windows (Hennekens 2012) and JUICE (Tichý 2012) [cf. Hennekens – Schaminée 2001, Tichý 2002] were used for the processing of phytosociological relevés.

3 RESULTS

3.1 Actual vegetation conditions of forests at Vysoké skalky

In fact, the uppermost part of the slopes of Vysoké skalky is not covered by stands of *Picea abies*. A substantial part of the cited elevations 1 000–1 050 m (see Introduction) is occupied by a compact summit rock, available without climbing only from the northwest side from a very short ridge with the lowest elevation 1 032 m. Above that only spurs of *Fagus sylvatica* forest reach, and the highest situated trees are of *Sorbus aucuparia*, *Fagus sylvatica* and *Acer pseudoplatanus* (with *Coryllus avellana*, *Sambucus racemosa*, *Salix caprea*, *Cotoneaster integerrimus*, *Ribes alpinum*).

Adjacent slopes on the Slovak part of the state border (running along the main ridge of the mountain range) are covered by climax deciduous forests dominated by *Fagus sylvatica*; *Acer pseudoplatanus* is admixed, I noticed saplings of *Abies alba* and also several *Picea abies* trees. Cultures of *Picea abies* spread below the summit of Vysoké skalky in lower altitudes only.

A similar picture appears on the Polish slopes of the area around the summit of the Vysoké skalky. Notable is a treeless gap to the northeast between summit rock walls and the upper forest line reaching around 1 029 m a.s.l. (49°22,833' N, 20°33,333' E, ± 6 m). Forest stands are dominated by *Fagus sylvatica* as demonstrated by phytosociological sample No. 1 (see Appendix).

To the east of the relevé plot No. 1, the field layer has a more grassy character (*Hordelymus europaeus*) and onward is without *Lunaria* (with *Aconitum moldavicum* [cf. Kučera 2011], *Cimifuga europaea*). Above the upper edge of the relevé plot No. 1 no stand of *Picea abies* grew in the past: located there is a group of three dead *Picea abies* trunks only, and several other *Picea* trees are scattered in the beech forest in the vicinity of the relevé No. 1.

Nevertheless, there is one stand of *Picea abies* close to the summit of Vysoké skalky (Figure 1: A). It is formed as an island inside of a beech forest and its upper limit reaches to elevation ca. 1 020 m (below the northwest ridge of the Vysoké skalky) and its lower limit to ca. 950 m. This stand was severely disturbed some years ago (before 2010) and today contains only a small portion of living *Picea abies* trees.

The herb layer and the entire spread of this former *Picea abies* island is marked by dominant *Rubus idaeus*, while a field layer of the adjacent beech areas under the summit ridge is made up of various vegetation types (*Galium odoratum*, *Asarum europaeum*, *Oxalis acetosella* [*Polygonatum verticillatum*, *Senecio ovatus*, sparsely *Rubus idaeus* of lower growth etc., also saplings of *Acer platanoides*] or *Dryopteris filix-mas*, *Galium odoratum*, *Lunaria rediviva* or below the summit *Lunaria rediviva*, *Dryopteris filix-mas* [*Lonicera nigra*, *Sambucus racemosa*, *Dentaria glandulosa*]). The most remarkable feature of the canopy of this *Picea abies* island (Figure 1: A) is the equal age of the trees (age class ca. 60–80 years): the stand is a planted monoculture. Also, the overall natural conditions (e.g. soil, climate) of mentioned *Picea*- vs. *Fagus*-dominated adjacent stands are more or less the same: the most important factor that differentiated the development of the undergrowth of the respective stands was the presence or absence of an anthropic influence = the plantation of *Picea abies*.

Similarly differentiated is another *Picea abies* island (Fig. 1: B) lying west of coordinates 49°22,859' N, 20°33,277' E (± 6 m, 1 027 m), with a lower elevation limit of 964 m (49°22,916' N, 20°33,197' E, ± 12 m). In the same manner as the previous one, it has artificial borders to the adjacent *Fagus* stands. This *Picea abies* stand-island was also planted by man less than ten decades ago (up to ca. 60–80 years). Part of the *Picea* trees was also wind-blown and another part died after a recent parasite infestation, but the rest *Picea* trees have survived. The area of the windthrow is overgrown by *Rubus idaeus* and *Senecio nemorensis* agg. Today there is admixed *Fagus sylvatica*, which rejuvenates (I noticed also a sapling of *Abies alba* in the stand's border). A potential rejuvenation of *Picea abies* is blocked here by rich secondary undergrowth: this subpopulation (as well as the previous one) will not survive a natural development of the vegetation cover.

Also the last of the *Picea abies* stands below the summit of Vysoké skalky on the Polish side of the state border slopes, neighbouring upon recent pastures (Figure 1: C, prevailing area between elevations 910–940 m), is a plantation, nowadays mostly destroyed by wind (*Rubus idaeus* is the dominant plant species recently).

Further apart from the summit of Vysoké skalky to the northwest, there are other *Picea abies* stands (Figure 1: D–E, F). Finally, in the area of the stand marked as 'D', I could confirm the data of Kulczyński (1928, p. 119) regarding the existence of *Picea abies* woodland with an herb layer dominated by *Vaccinium myrtillus*, with occurrence of *Homogyne alpina* and also *Vaccinium vitis-idaea*. Its elevation range is ca. 870–940 m. This unique vegetation is induced by thin soil cover developing on special bedrock – carbonate hill debris with boulders frequently reaching dimensions of 40 × 40 cm. Debris boulders are not weathering much so the corresponding habitat is very conservative. Uprooting of trees reveals bare boulders of hill debris.

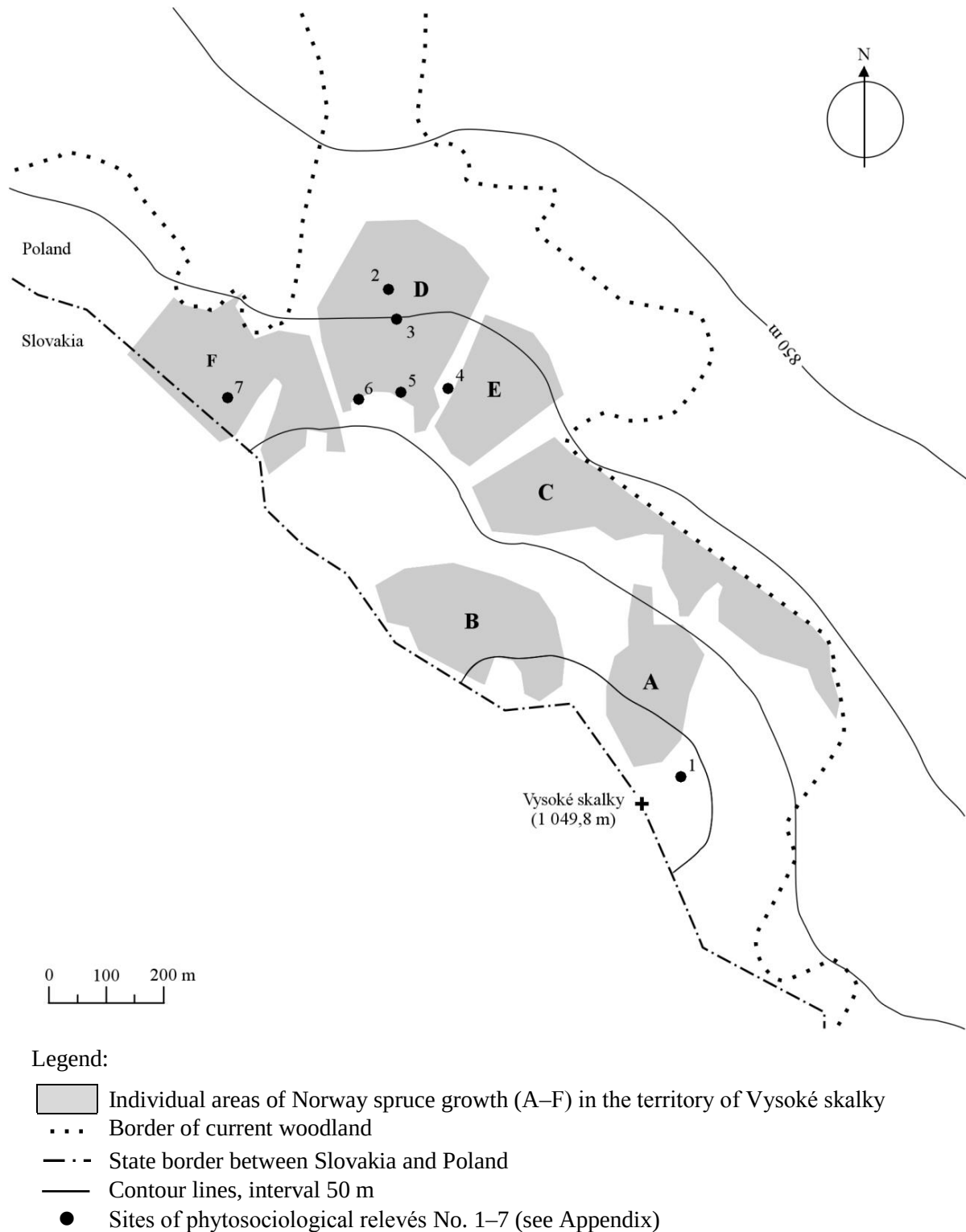


Figure 1. Sketch map of the locality

However, also here a great part of *Picea abies* population was attacked by parasites (before 2010) and died. Insect attack was most probably preceded by wind disturbance of this and other stands in the vicinity. However, a sufficient amount of *Picea* trees resisted and survival of this population is ensured by rich natural rejuvenation of Norway spruce with variable age spans, and forming thickets here and there. An example of the vegetation type is given by the phytosociological relevé No. 2. The lateral border of *Vaccinium myrtillus*-*Homogyne alpina*-

Picea abies woodland to the former original beech forest is indicated by typical herbaceous facies of beech woodlands (*Galium odoratum* [also dominant], *Asarum europaeum*, *Symphytum cordatum*, or *Astrantia major*, *Petasites albus*; also *Cicerbita alpina* and *Streptopus amplexifolius* grow here (site of the last species: 49°23,001' N, 20°33,097' E, ± 7 m, 930 m)) and the absence of *Vaccinium myrtillus*. The background here at the hill debris border could also be formed by carbonate debris, but the size of the rocks is considerably smaller and the soil profile is deeper than in habitat of *Vaccinium myrtillus*-*Homogyne alpina*-*Picea abies* woodland.

Above the upper right corner of the relevé plot No. 2, vegetation with *Hypericum maculatum*, *Deschampsia cespitosa*, *Agrostis capillaris*, *Taraxacum* sp. is growing. This occurrence indicates an influence of the former pasture.

According to Kulczyński (1928, p. 118–119), this area is home of a native *Picea abies* woodland. However, despite the special habitat conditions, trees of *Fagus sylvatica* could also grow and prosper here and could withstand windstorms. In the vicinity of the relevé plot No. 2, an older *Fagus* tree (more than 60–70 years) is growing and it is a source for (microlocally considerably rich) the rejuvenation of *Fagus*. Such a vegetation sample is given in relevé No. 3, spatially only 10 m from the relevé plot No. 2. The prevailing part of the *Picea* trees on the plot was windblown, other parts died after parasite attack; *Fagus* grow successfully.

However, even *Fagus* can dominate in the canopy in this debris territory. The next relevé (No. 4) was recorded on the lateral debris ridge, with rocks on the surface. A different vegetation type is developed here.

To the east of the relevé plot at the slope beginning down from this lateral ridge, hill debris and communities with *Vaccinium myrtillus* cover the slope in the same manner as west of the ridge (Figure 1: D). *Lonicera nigra* is abundant, rejuvenation of *Picea abies* is rich under the parasite-attacked and wind-blown *Picea* trees (Figure 1: E). Also, an old *Abies alba* tree was recorded (below coordinates 49°22,986' N, 20°33,224' E, ± 9 m, 930 m a.s.l., here also an example of secondary, temporary occurrence of *Cotoneaster integerrimus*, see in Discussion): this tree species should be included among species that once co-determined the original woodland type. Secondary succession is here in a more advanced phase as in the stand marked in the Figure 1 as 'D'.

The original course of the upper limit of the local natural occurrence of woodland with abundant *Picea abies* is hard to determine today. *Picea abies* was propagated by human intervention directly (plantation) or indirectly (spreading by secondary succession). Relevé plot No. 5 is probably one of the highest points mixed woodland with *Picea abies* could reach originally. Today, a great part of the former *Picea* stand has been destroyed – however, its population will persist as the species has rejuvenated long ago. An older *Fagus* tree uphill above the relevé plot is the origin of the numerous beech saplings (with cover-abundance value 4 [–5]), a different habitat is indicated there by *Sanicula europaea* (richly), *Primula elatior*, *Symphytum cordatum*, *Ranunculus platanifolius*, *Mercurialis perennis*, *Galium odoratum* (*Pulmonaria obscura*). Originally there was already abeech woodland developed here.

The recent vegetation cover in the western uppermost part of the *Picea* stand 'D' is interesting. Under dead *Picea* trunks, a rich undergrowth of *Lonicera nigra* and *Vaccinium myrtillus* grows. The slope relief indicates that hill debris is still 'living'. In the geological history, hill debris of 'stand D' probably originated through the collapse of solid rock. In the uppermost, rocky part of the hill debris, with formation of small rock walls and falling rocks and boulders (in the other place, we have seen also a small vertical cavern: 49°22.953' N, 20°33.167' E, ± 7 m), wind and parasite disturbance caused all mature *Picea* specimens to die out and only young ones are living. However, the *Fagus* population here (relevé plot No. 6)

could also grow successfully, supplemented with rejuvenation from trees growing uphill. After *Picea* died out, treeless scrub vegetation partly developed. The uppermost edge of the hill debris begins slightly higher. There, under tall *Fagus* trees, a *Vaccinium myrtillus* population is still growing, but only of a dwarfed growth. Further uphill there is distributed typical beech vegetation of deeper calcareous soils.

The last *Picea abies* stand in the Polish part of the territory of Vysoké skalky, marked on Figure 1 by the letter 'F', is again a plantation. It continues directly to a younger spruce plantation (age 40–60 years) on the Slovak part of the state border. Relevé No. 7 shows clearly striking differences in the herb layer in comparison to relevés 2, 3 and 5. Also, non-indigenous species *Larix decidua* was planted here.

3.2 Formal comments on the association *Polysticho-Piceetum* and data on its occurrence at Vysoké skalky

The correct association name completed according to Recomm. 10C of the International Code of Phytosociological Nomenclature (ICPN: Weber et al. 2000) is *Polysticho lonchitidis-Piceetum* W. Matuszkiewicz ex J. Matuszkiewicz 1977 (see Kučera 2010, who also designated a lectotype to the association). Yet, phytocoenoses of Kulczyński's *Vaccinium myrtillus-Homogyne alpina-Picea abies* woodland (see above) do not correspond to this syntaxon as well as to its probable syntaxonomical synonym *Oxalido acetosellae-Piceetum* Březina et Hadač in Hadač et al. 1969.

Also questionable is the utilisation of Kulczyński's (1928) phytosociological data on '*Piceetum excelsae*' as a phytosociological relevé; for example, in the synthesis of J. Matuszkiewicz (1977). In contrast to other species lists in the work of Kulczyński, the species listing on pages 119–120 (Kulczyński 1928) should not be accepted as a constancy table nor as a single relevé by reason that it is a list of all species found in the whole area of the occurrence of the *Picea abies* stand extending up to 'several hectares' (see Kulczyński 1928, p. 119), only accompanied with cover-abundance values. Therefore, the name proposed by Kulczyński – i.e. *Piceetum excelsae* Kulczyński 1928 – is to be nomenclaturally treated not only as a nomen illegitimum (ICPN Art. 31), but also primarily as a nomen invalidum (Art. 2b → Art. 7).

4 DISCUSSION

As shown above, there exist more than one *Picea abies* stand in the territory of Vysoké skalky (Figure 1: A–F). The elevation range of both *Picea abies* stands 'A' and 'B', reaching continuously down to 950 m/964 m, considerably exceed the altitudinal limits of 1 000–1 050 m given by Matuszkiewicz (1982, 2014) and Szelağ (1995) for natural occurrence of a *Polysticho-Piceetum* stand. As no such *Picea abies* stands on the slopes of Vysoké skalky exist, these published data should be taken as incorrect. In addition, occurrence of several *Picea abies* trees in the vicinity of the relevé No. 1 could not be the reason for the publishing of such data on occurrence of a native Norway spruce woodland in the summit area of Vysoké skalky.

In respect to elevation range of the stands 'A' and 'B', more closely fitting would be the information of Pancer-Kotejowa et al. (1982, p. 319: around 1 000 m), which could be applied to both *Picea abies* stands under consideration (Figure 1: A, B). Nonetheless, their species composition is *completely different* from the phytosociological data of Kulczyński (1928, see p. 119–120). The same stands for the species composition of *Picea* stands are labeled in Figure 1 as 'C' and 'F'.

On the other hand, the real existence of a *Vaccinium myrtillus*-*Homogyne alpina*-*Picea abies* community in the territory of Vysoké skalky (Kulczyński 1928) was indeed confirmed (see relevé No. 2, within the *Picea abies* stand 'D'). Field occurrence of this vegetation type corresponded exactly to the place of *Piceetum excelsae* indicated by Kulczyński (1928) in his phytosociological map of the Pieniny. Kulczyński (1928, p. 119) is at the same time almost the only one who published correct information on its distribution; the second person was Zarzycki (1981, 1982) with elevation data 900–950 m resp. 890–950 m.

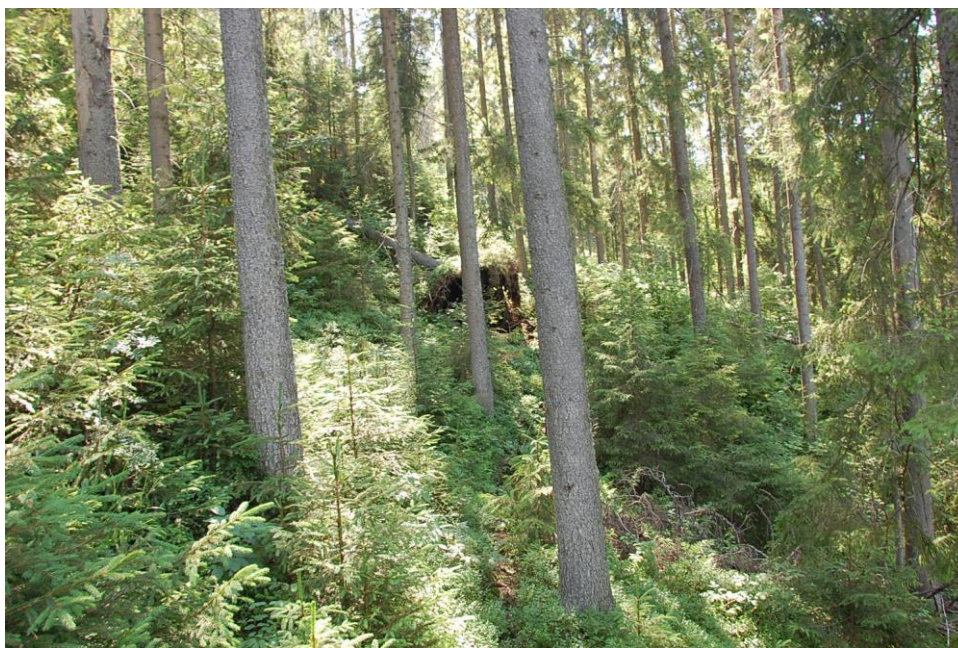


Figure 2. Plant cover in the site of the relevé No. 2.

Among all other *Picea* stands of Vysoké skalky, the area of the stand 'D' is the one that provides a sustainable existence of the *Picea abies* population, including interspecific competition. This is due to a special conservative debris habitat. In my opinion, the stand marked in the map (Figure 1) as 'E' is also an example of the refugium habitat of the *Picea abies* population: the total area of the natural occurrence of *Picea abies* in the territory of Vysoké skalky is thus larger than Kulczyński (1928) suggested in his phytosociological map.

However, the phytosociological sample No. 3 confirms that the locality described by Kulczyński (1928, p. 119) is not an example of the authentic *Picea abies* forest. The situation of species composition described by Kulczyński (1928) has its origin in long-term historic land use, deforestation of the prevalent territory of slopes of Malé Pieniny mountain ridge, and changes in species composition of woodland rests. Also, the species list of Kulczyński (1928) confirms the appearance of species with human-influenced distribution, 'aliens' in the originally common woodlands of this area: *Antennaria dioica*, *Hypericum maculatum*, *Potentilla erecta*, *Cruciata glabra* (*Veronica chamaedrys*; *Cotoneaster integerrimus* from secondary, temporary lighting of the stand).

Recent development of vegetation cover point to more-balanced mixed stands of *Picea abies* and *Fagus sylvatica* in the future. Thus, in contrast to Kulczyński (1928) and data of other authors on the natural existence of an autochthonous *Picea abies* plant community (see Introduction) in the territory of Vysoké skalky, I am of the opinion that the existence of *Picea abies* in this locality should be evaluated more likely as an isolated local relict of the *Picea abies* population persisting within forest of mixed tree species composition. Constant special habitat conditions are confirmed by remarkable co-occurrence of dominant *Vaccinium*

myrtillus and *V. vitis-idaea* at the first place, *Homogyne alpina* and *Calamagrostis villosa*. In contrast to all other *Picea* stands (plantations) in the territory of Vysoké skalky, a gradation of *Rubus idaeus* does not occur.

5 SIGNIFICANCE OF *PICEA ABIES* OCCURRENCE AT VYSOKÉ SKALKY

If the population of *Picea abies* of the stands 'D' and 'E' (Figure 1) is really a native (thus relict) population, then it could have developed most likely a unique, isolated gene pool. Genetic studies of possible differences compared to the closest native stands (Tatry Mts) should be considered. *Picea abies* forests predominated in the landscape of northern Slovakia in early Holocene (cf. Jankovská 1991, 1998). In the lower mountain ranges adjacent to the Tatry Mts there is reconstructed a potential natural vegetation consisting of *Abies-Fagus* forests (cf. Kučera 2012a, b; see also Kučera 2015): the *Picea abies* woodland of Vysoké skalky in the Malé Pieniny Mts was separated from the closest native *Picea abies* populations in the Tatry Mts during the Holocene (cf. Krippel 1963, 1986) by natural beech and fir-beech forests of the territory of Pieniny and the Spišská Magura Mts. Current Norway spruce stands covering large areas of both mountain ranges are results of long-term anthropic influence (see below).

Long-term isolation of the *Picea abies* population of Vysoké skalky could also lead to a particular adaptation to ecological conditions of the lower montane vegetation zone, which has special importance for forestry. However, the existence of Norway spruce cultures of uncertain provenience in the vicinity of *Picea abies* population of the stands 'D' and 'E' is a threat to preservation of the supposed original genotypic and phenotypic features.

Picea abies individuals and stands originated by plantation and saplings/young trees from their rejuvenation should be strictly regulated above all in the area of the Nature Reserve Wysokie Skalki. Also, spruce plantations west of this nature reserve should not directly influence the genetic composition of the above-mentioned relict population.

6 REMARKS TO *PICEA ABIES* OCCURRENCE IN THE SURROUNDINGS OF VYSOKÉ SKALKY

In the wider surroundings, we have also noticed a recent plantation of Norway spruce (cf. also Pawłowski 1925). However, as we can see in the territory of Vysoké skalky, plantations of Norway spruce are very vulnerable cultures in these low altitudes of the Malé Pieniny. Economic profit here is insecure because of the lability of tree stands and strong predisposition of *Picea abies* stands to disturbance (even total elimination) by wind and insect attacks.

Besides the plantation of Norway spruce, on the recent borders of pastures to forest in the Malé Pieniny, easy rejuvenation of *Picea abies* is in progress here and there. This is a typical form of a secondary succession, a spontaneous process of the spreading of forest back to its original place. In the lower altitudes, in a lower montane altitudinal vegetation belt, the first and long-lasting stage (in several phases) is formed by *Picea abies* (cf. Myczkowski – Grabski 1962) if a 'parent' *Picea abies* stand (plantation) is in the surroundings. A secondary succession of *Fagus sylvatica* in such secondary, substitutional *Picea* stands could only be seen much later (cf. Kučera 2012a, chapter 4). However, long-term natural competition between *Picea abies* and other plant species generally results in the elimination of *Picea abies* in the lower montane belt. It seems that this rule has the one and only exception in the

whole region of the Malé Pieniny – a population of *Picea abies* within the Kulczyński's (1928) stand of '*Piceetum excelsae*' is maintained by special habitat conditions.

Except for such special cases, anthropogenic changes of the vegetation cover (direct or indirect) are the reason of the occurrence or even dominance of *Picea abies* in forest stands of the Pieniny Mts. According to my evaluation (cf. Kučera 2007, 2012b), stands evaluated as *Piceo-Abietum carpaticum* by Kulczyński (1928) also originated by a spontaneous secondary succession, related to the planting of *Picea abies* (or direct plantation establishment) and the historical land use in the centuries before 1900 (see distribution of this plant community on his phytosociological map and extent of deforested area in the early 20th century); nomenclatural status of the name: *Piceo-Abietetum carpaticum* Kulczyński 1928, nom. inval., ICPN Art. 3e (Weber et al. 2000). In the adjacent mountain range of the Spišská Magura, superiority of *Fagus sylvatica* (*Abies alba*) in the natural development of forest vegetation is confirmed up to the uppermost elevation (1 259 m) (Kučera 2012b). Equivalently, the occurrence of a natural *Picea abies* forest zone is not expected in the other adjacent mountain range of the Beskid Sądecki (Kučera 2012a), although development of a such altitudinal vegetation zone was presumed there by Pawłowski (1925) already above elevation 1 100 –1 130 m and by Myczkowski – Grabski (1962) above (1 000) 1 050 or 1 100 m; Fabijanowski (1962) expected typical *Picea* forests even at altitudes 780 m and 915 m.

7 CONCLUSIONS

According to the field research in the region of Vysoké skalky with data on the occurrence of *Piceetum excelsae* published by Kulczyński (1928) I conclude:

1. Almost all stands of *Picea abies* on the slopes of Vysoké skalky are plantations – cultures unstable to environmental influences.
2. Only one local *Picea abies* stand (Figure 1: D, E) corresponds to the phytosociological data of Kulczyński (1928).
3. Data of altitudinal range of the occurrence of *Picea* stand corresponding to Kulczyński's data published by Matuszkiewicz (1982), Pancer-Kotejowa et al. (1982, p. 319) and Szeląg (1995) are incorrect.
4. The plant community of the Kulczyński's *Picea* stand is not a native *Picea abies* woodland, and should probably be evaluated as a natural, isolated refugium of occurrence of a *Picea abies* population within a mixed forest stand.
5. Nature conservation should consider an appropriate management in respect of the preservation of this presumably unique gene pool of *Picea abies* in the Malé Pieniny.

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APPENDIX

Phytosociological relevés recorded in the region of Vysoké skalky

Relevé No. 1: 49°22.826' N, 20°33.360' E, ± 6 m, 1 023 m a.s.l. (upper limit), steep slope, aspect NE (46 °), relevé area 20 × 20 m, total cover 100%, E₃ 95%, E₂ 15%, E₁ 80%, E₀ 0%, rocks 1%, wood 0,5%, *Fagus* trees above 80–100 years, *Fagus* perimeter_{1.3} [p.] 149 cm, height (h.) [29] m, age up to 80 years, 18. 7. 2011, P. Kučera, R. Rapant (PK220).

E₃: *Fagus sylvatica* 5, *Acer pseudoplatanus* 1, *Picea abies* 1,

E₂: *Fagus sylvatica* 2b, *Corylus avellana* r,

E₁: *Acer pseudoplatanus* +, *Fagus sylvatica* +, *Lonicera xylosteum* r, *Picea abies* r, *Ribes alpinum* r, *R. uva-crispa* r,

Lunaria rediviva 3, *Galium odoratum* 2b, *Dryopteris filix-mas* 1, *Galeobdolon montanum* 1, *Geranium robertianum* 1, *Hordelymus europaeus* 1, *Hylotelephium argutum* 1, *Mercurialis perennis* 1, *Oxalis acetosella* 1, *Poa nemoralis* 1, *Roegneria canina* 1 (det. D. Bernátová), *Symphytum cordatum* 1, *Aconitum variegatum* +, *Actaea spicata* +, *Adoxa moschatellina* +, *Asarum europaeum* +, *Bromus benekenii* + (det. D. Bernátová), *Campanula trachelium* +, *Dentaria glandulosa* +, *Epilobium montanum* +, *Galeopsis speciosa* +, *Geranium sylvaticum* +, *Glechoma hirsuta* +, *Hypericum hirsutum* +, *Impatiens noli-tangere* +, *Lamium maculatum* +, *Lathyrus vernus* +, *Milium effusum* +, *Myosotis* sp. +, *Paris quadrifolia* +, *Polygonatum verticillatum* +, *Polystichum aculeatum* +, *Ranunculus platanifolius* +, *Rubus idaeus* +, *Senecio ovatus* +, *Silene dioica* +, *Stachys sylvatica* +, *Thalictrum aquilegiifolium* +, *Viola reichenbachiana* +, *Calamagrostis varia* r, *Carduus personata* r, *Cicerbita alpina* r, *Cirsium erisithales* r, *Dryopteris carthusiana* r, *Petasites albus* r, *Primula elatior* r, *Ranunculus lanuginosus* r, *Salvia glutinosa* r, *Tithymalus amygdaloides* r, *Urtica dioica* r, *Veronica officinalis* r.

Relevé No. 2: 49°23.046' N, 20°33.163' E, ± 8 m, 890 m a.s.l., aspect variable (NNW, 328 °), total cover 100%, E₃ 45%, E₂ 20%, E₁ 93%, E₀ 20%, fallen wood 1%, age of *Picea* trees up to 80 r., *Picea* p. 96 cm, h. [22] m, age up to cca 80 years, 17. 7. 2011, P. Kučera, R. Rapant (PK217).

E₃: *Picea abies* 3,

E₂: *Picea abies* 2b (in spots), *Lonicera nigra* 1, *Fagus sylvatica* +, *Salix caprea* +, *Salix* × *subcaprea* r,

E₁ *Picea abies* 2a, *Lonicera nigra* 1, *Acer pseudoplatanus* +, *Fagus sylvatica* +, *Ribes petraeum* +, *Salix caprea* +, *Sorbus aucuparia* +, *Abies alba* r, *Corylus avellana* r,

E₁ A: *Vaccinium myrtillus* 4, *Homogyne alpina* 2b, *Rubus idaeus* 2a, *Dryopteris dilatata* 1, *Gymnocarpium dryopteris* 1, *Lycopodium annotinum* 1, *Maianthemum bifolium* 1, *Oxalis acetosella* 1, *Valeriana tripteris* 1, *Avenella flexuosa* +, *Calamagrostis villosa* +, *Luzula luzuloides* +, *L. sylvatica* ssp. *sylvatica* +, *Orthilia secunda* +, *Poa nemoralis* +, *Vaccinium vitis-idaea* +, *Deschampsia cespitosa* r, *Urtica dioica* r,

E₁ B: *Agrostis capillaris* +, *Athyrium filix-femina* +, *Chrysosplenium alternifolium* +, *Fragaria vesca* +, *Gentiana asclepiadea* +, *Paris quadrifolia* +, *Senecio ovatus* +, *Alchemilla* sp. r, *Calamagrostis varia* r, *Dentaria glandulosa* r, *Geum rivale* r (lower border of the relevé plot), *Epilobium montanum* r, *Primula elatior* r, *Prunella vulgaris* r, *Streptopus amplexifolius* r, *Veronica officinalis* r,

E₀: *Dicranum scoparium* 2a, *Polytrichum formosum* 1, *Sphagnum quinquefarium* 1, *Plagiochila porelloides* +, *Plagiomnium affine* +, *Plagiothecium denticulatum* +, *Tritomaria quinquedentata* +, *Calypogeia muelleriana* +, *Cephalozia bicuspidata* +, *Eurhynchium angustirete* +, *Lepidozia reptans* +.

Inside of the chosen relevé plot (of standard size 20 × 20 m) a slightly different microhabitat (E₁ B, more wet and nutrient, with two fallen *Picea* trunks) fell within in the lower part of the plot.

Relevé No. 3: 49°23.031' N, 20°33.169' E, ± 8 m, 900 m a.s.l., aspect variable (NNW, 347 °), relevé area 15 × 20 m, total cover 100%, E₃ 40%, E₂ 18%, E₁ 90%, E₀ 10%, fallen wood 2%, *Picea* p. 121 cm, h. [22,5] m, age up to 60–80 years, *Fagus* trees (at left bottom part) age up to 30 years, h. 6 (7) m, 17. 7. 2011, P. Kučera, R. Rapant (PK218).

E₃: *Fagus sylvatica* 2b, *Picea abies* 2b (before disturbance 3),

E₂: *Picea abies* 2a, *Corylus avellana* 1, *Fagus sylvatica* 1, *Acer pseudoplatanus* +, *Sorbus aucuparia* +, *Abies alba* r,

E₁: *Lonicera nigra* 1, *Picea abies* 1, *Acer pseudoplatanus* +, *Daphne mezereum* +, *Fagus sylvatica* +, *Rosa pendulina* +, *Salix subcaprea* +, *Sorbus aucuparia* +, *Abies alba* r,

Vaccinium myrtillus 2b, *Calamagrostis arundinacea* 2a, *Rubus idaeus* 2a, *Athyrium filix-femina* 1, *Calamagrostis varia* 1, *C. villosa* 1, *Dryopteris filix-mas* 1, *Galeobdolon montanum* 1, *Galium odoratum* 1, *Gentiana asclepiadea* 1, *Homogyne alpina* 1, *Oxalis acetosella* 1, *Senecio ovatus* 1, *Valeriana tripteris* 1, *Clematis alpina* +, *Dryopteris dilatata* +, *Fragaria vesca* +, *Galium rotundifolium* +, *Gymnocarpium dryopteris* +, *Luzula luzuloides* +, *Maianthemum bifolium* +, *Mycelis muralis* +, *Prenanthes purpurea* +, *Rubus fruticosus* agg. +, *Agrostis capillaris* r, *Avenella flexuosa* r, *Chamerion angustifolium* r, *Deschampsia cespitosa* r, *Epilobium montanum* r, *Hieracium* sp. r, *Hypericum maculatum* r, *Urtica dioica* r, *Vaccinium vitis-idaea* r, *Veronica officinalis* r.

E₀: *Eurhynchium angustirete* 1, *Dicranum scoparium* 1, *Barbilophozia barbata* +, *Plagiochila porelloides* +, *Plagiomnium affine* + *Sphagnum quinquefarium* +.

Relevé No. 4: 49°22.999' N, 20°33.208' E, ± 14 m, 927 m a.s.l., gentle slope, aspect NNE (27 °), relevé area 20 × 20 m, total cover 98%, E₃ 70%, E₂ 25%, E₁ 75%, bryophytes only on rocks, rocks 8%, *Fagus* trees (up to) 60–80 years, *Fagus* h. above 20 m (also *Acer*), age up to 70–80 years, 17. 7. 2011, P. Kučera, R. Rapant (PK219).

E₃: *Fagus sylvatica* 4, *Acer pseudoplatanus* 2b, *Picea abies* 1,

E₂: *Corylus avellana* 2b, *Fagus sylvatica* 2a, *Lonicera nigra* +,

E₁: *Fagus sylvatica* (1) 2a, *Acer pseudoplatanus* 1, *Fraxinus excelsior* +, *Lonicera nigra* +, *Picea abies* +, *Rosa pendulina* +, *Abies alba* r, *Sambucus racemosa* r, *Sorbus aucuparia* r, *Viburnum opulus* r,

E₀: *Galium odoratum* 2b, *Dryopteris filix-mas* 2a, *Calamagrostis varia* 1, *Galeobdolon montanum* 1, *Melica nutans* 1, *Oxalis acetosella* 1, *Rubus fruticosus* agg. 1, *Vaccinium myrtillus* 1, *Valeriana sambucifolia* 1 (det. D. Bernátová), *Brachypodium sylvaticum* +, *Calamagrostis arundinacea* +, *Chaerophyllum hirsutum* +, *Clinopodium vulgare* +, *Cruciata glabra* +, *Dentaria glandulosa* +, *Epilobium montanum* +, *Festuca* sp. +, *Fragaria vesca* +, *Galium anisophyllum* +, *Hordelymus europaeus* +, *Hypericum maculatum* +, *Luzula luzuloides* +, *Maianthemum bifolium* +, *Mycelis muralis* +, *Paris quadrifolia* +, *Poa nemoralis* +, *Polypodium vulgare* +, *Polystichum aculeatum* +, *Prenanthes purpurea* +, *Primula elatior* +, *Ranunculus platanifolius* +, *Rubus idaeus* +, *Sanicula europaea* +, *Senecio ovatus* +, *Valeriana tripteris* +, *Viola reichenbachiana* +, *Ajuga reptans* r, *Athyrium filix-femina* r, *Epipactis helleborine* r, *Hylotelephium maximum* r, *Polygonatum verticillatum* r, *Urtica dioica* r, *Veronica chamaedrys* r.

Outside of the relevé plot at the slope beginning down from this lateral ridge: *Lonicera xylosteum*, *Ribes alpinum*.

Relevé No. 5: 49°22.998' N, 20°33.170' E, ± 6 m, 935 m a.s.l., steep slope, aspect NNE (25 °), relevé area 20 × 20 m, total cover 100%, E₃ 40% (formerly above 75%), E₂ 55%, E₁ 80%, E₀ cca 15%, *Fagus* tree probably above 80 years, h. 16–17 m, *Picea* trees were up to 60–80 years old, most of them are dead, *Picea* h. above 20 m (21–22 m), 18. 7. 2011, P. Kučera, R. Rapant (PK223).

E₃: *Picea abies* 3, *Fagus sylvatica* 2a (2b), *Abies alba* 1 (young), *Acer pseudoplatanus* 1 (young),

E₂: *Lonicera nigra* 3, *Corylus avellana* 2a, *Fagus sylvatica* 2a, *Picea abies* 2a, *Sorbus aucuparia* ssp. *aucuparia* 1, *Abies alba* +, *Acer pseudoplatanus* +,

E₁ (partly mixed types): *Picea abies* 2a, *Lonicera nigra* 1, *Abies alba* +, *Acer pseudoplatanus* +, *Fagus sylvatica* +, *Ribes alpinum* +, *Sorbus aucuparia* +, *Corylus avellana* r, *Salix silesiaca* r,

Vaccinium myrtillus 3, *Galium odoratum* 2a, *Gymnocarpium dryopteris* 2a, *Calamagrostis varia* 1, *Dryopteris dilatata* 1, *Hordelymus europaeus* 1, *Galeobdolon montanum* 1, *Oxalis acetosella* 1, *Rubus idaeus* 1, *Vaccinium vitis-idaea* 1, *Valeriana tripteris* 1, *Asplenium viride* +, *Athyrium filix-femina* +, *Chamerion angustifolium* +, *Dentaria glandulosa* +, *Dryopteris filix-mas* +, *Epilobium montanum* +, *Fragaria vesca* +, *Maianthemum bifolium* +, *Petasites albus* +, *Phegopteris connectilis* +, *Poa nemoralis* +, *Polystichum aculeatum* +, *Senecio ovatus* +, *Pyrethrum clusii* +, *Viola reichenbachiana* +, *Avenella flexuosa* r, *Carex digitata* r, *Clematis alpina* r, *Cystopteris fragilis* r, *Hieracium* sp. r, *Hypericum maculatum* r, *Mercurialis perennis* r, *Ranunculus platanifolius* r, *Sanicula europaea* r, *Streptopus amplexifolius* r, *Urtica dioica* r,

E₀: *Rhizomnium punctatum* 1, *Eurhynchium angustirete* 1, *Dicranum scoparium* 1, *Blepharostoma trichophyllum* +, *Calypogeia muelleriana* +, *Cephalozia bicuspidata* +, *Lepidozia reptans* +, *Lophozia ventricosa* +, *Plagiochila asplenoides* +, *Polytrichum formosum* +, *Sphagnum quinquefarium* +, *Tetraphis pellucida* +.

Relevé No. 6: 49°22.996' N, 20°33.143' E, ± 5 m, 940 m a.s.l., slope variable (total [40] °), aspect N (350 °), relevé area 20 × 20 m, total cover 97%, E₃ (68%), E₂ (35%), E₁ (40%), E₀ (35% everywhere on rocks), *Fagus* h. even 20 m, *Picea* h. above 20 m, 18. 7. 2011, P. Kučera, R. Rapant (PK222).

E₃: *Fagus sylvatica* 4, *Picea abies* 2b [formerly 3],

E₂: *Lonicera nigra* 2b, *Ribes alpinum* 2a, *Corylus avellana* 1, *Picea abies* 1, *Salix caprea* 1, *Sambucus racemosa* 1, *Sorbus aucuparia* +, *Lonicera xylostium* r, *Salix* × *subcaprea* r,

E₁: *Sambucus racemosa* 1, *Ribes alpinum* 1, *Lonicera nigra* 1, *Sorbus aucuparia* +, *Rosa pendulina* +, *Acer pseudoplatanus* +, *Picea abies* r, *Abies alba* r,

Gymnocarpium dryopteris 2a, *Oxalis acetosella* 2a, *Geranium robertianum* 2a, *Dryopteris filix-mas* 1, *Galeobdolon montanum* 1, *Galium odoratum* 1, *Rubus idaeus* 1, *Urtica dioica* 1, *Vaccinium myrtillus* 1, *Athyrium filix-femina* +, *Brachypodium sylvaticum* +, *Cardamine flexuosa* +, *Cystopteris fragilis* +, *Dryopteris dilatata* +, *Epilobium montanum* +, *Fragaria vesca* +, *Hylotelephium argutum* +, *Luzula luzuloides* +, *Paris quadrifolia* +, *Poa nemoralis* +, *Polypodium vulgare* +, *Polystichum aculeatum* +, *Senecio ovatus* +, *Valeriana tripteris* +, *Actaea spicata* r, *Asplenium trichomanes* r, *A. viride* r, *Cardaminopsis arenosa* r, *Dentaria glandulosa* r, *Dryopteris expansa* r, *Valeriana sambucifolia* r.

Relevé No. 7: 49°22.993' N, 20°33.053' E, ± 5 m, 932 m a.s.l., gentle slope, aspect NNW/N (348 °), relevé area 20 × 20 m, total cover 97%, E₃ 68%, E₂ 2%, E₁ 95%, E₀ cca 15%, wood 5%, *Picea* trees 60–80 years, *Picea* p. 129 cm, h. [25,5] m, age 60–80 r., 18. 7. 2011, P. Kučera, R. Rapant (PK221).

E₃: *Picea abies* 4, *Larix decidua* r,

E₂: *Fagus sylvatica* +, *Acer pseudoplatanus* r, *Fraxinus excelsior* r, *Lonicera nigra* r,

E₁: *Fagus sylvatica* 1, *Acer pseudoplatanus* +, *Fraxinus excelsior* +, *Abies alba* r, *Lonicera nigra* r, *Sambucus racemosa* r, *Sorbus aucuparia* r,

Rubus idaeus 3, *Senecio ovatus* 3, *Oxalis acetosella* 2b, *Galeobdolon montanum* 2a, *Asarum europaeum* 1, *Athyrium filix-femina* 1, *Dryopteris dilatata* 1, *D. filix-mas* 1, *Galium rotundifolium* 1, *Geranium robertianum* 1, *Lysimachia nemorum* 1 (det. D. Bernátová), *Adoxa moschatellina* +, *Cardamine flexuosa* + (det. D. Bernátová), *Festuca gigantea* +, *Galium odoratum* +, *Mercurialis perennis* +, *Mycelis muralis* +, *Poa nemoralis* +, *Primula elatior* +, *Silene dioica* +, *Urtica dioica* +, *Viola reichenbachiana* +, *Actaea spicata* r, *Chamerion angustifolium* r, *Epilobium montanum* r, *Fragaria vesca* r, *Hylotelephium argutum* r, *Platanthera bifolia* r, *Prenanthes purpurea* r, *Roegneria canina* r (det. D. Bernátová), *Salvia glutinosa* r.

E₀: *Brachythecium reflexum* 1, *B. starkei* 1, *Rhizomnium punctatum* 1, *Dicranum scoparium* +, *Plagiothecium denticulatum* +, *Plagiomnium affine* +.

Micropropagation of Leuce-poplars and evaluation of their development under sandy site conditions in Hungary

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Abstract – Leuce-poplars are a native stand-forming tree species throughout Hungary. Several species or selections of them are used as ornamental plants in parks or to line streets and highways. They cover approximately 4.0 per cent of the total forested area in Hungary (70 000 ha). The white (grey) poplar belongs to the Leuce poplars and plays a significant role in sand fixation, regional forestation, and nature conservation. The National Agricultural Research and Innovation Centre, Forest Research Institute or NARIC-FRI (formerly known as the Forest Research Institute) is involved in long-term breeding work for the selection of fast-growing white poplar trees under dry conditions. In vitro multiplication of trees is applied mainly to fruit growing trees in Hungary; in forestry research it is used primarily for selective breeding. This paper presents a short overview of the most important issues concerning the biotechnology of different *Populus* species, the related research on micropropagation trials, and the results of field investigations of micropropagated Leuce-poplar clone experiments.

***Populus* sp. / biotechnology / in vitro / rejuvenation / selected clones / field trials**

Kivonat – Leuce-nyárok mikroszaporítása és homokterületen történő termesztésük kiértékelése **Magyarországon**. A Leuce-nyárok őshonos állományalkotó fajok Magyarországon. Néhány fajukat, illetve szelekciójukat parkokban, utcák, utak mentén díszfaként használják. Magyarországon az összes erdőszűlt területnek kevesebb mint 4%-át teszik ki (70 000 ha). A Leuce-nyárokhoz tartozó fehér (szürke) nyár jelentős szerepet játszik a homoktalajok megkötésében, azok erdősítésében, és természetvédelmi szempontból is fontos. Száraz termőhelyi viszonyokra szelektált, gyorsan növekvő Leuce-nyárok hosszútávú nemesítési munkája folyik a - korábban Erdészeti Tudományos Intézet, ma már - a Nemzeti Agrárkutatási és Innovációs Központ Erdészeti Tudományos Intézetében (NAIK-ERTI). Magyarországon fás növények esetében in vitro szaporítási eljárásokat főleg a gyümölcsstermesztésben alkalmaznak, az erdészeti kutatásban elsősorban a szelekciós nemesítés során használják. Jelen tanulmány rövid áttekintést nyújt a különböző nyár fajok legfontosabb biotechnológiai vonatkozásairól, a kapcsolódó mikroszaporítási kísérletekről, valamint mikroszaporítással előállított Leuce-nyár klónok termesztési kísérleteinek eredményeiről.

***Populus* sp. / biotechnológia / in vitro / juvenilizáció / szelektált klónok / terepi kísérletek**

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1 INTRODUCTION

Inside the section of Leuce poplars, the white poplar (*Populus alba* L.), and its most important natural hybrid the grey poplar, (*Populus* × *canescens*) are native to Hungary. The area of native poplar stands and plantations was about 77 000 ha in 2012 (4.0 per cent of the total forested land), with a standing volume of 13.0 million m³ (169 m³ ha⁻¹).

The NARIC Forest Research Institute is the most important national institution for poplar breeding and improvement. Investigations of selected individuals and populations of native poplars laid the foundation for their in situ and ex situ conservation.

Clonal selection is very effective on additive and non-additive genetic components. Asexual propagation is extremely important for the conservation of selected genotypes. Traditional vegetative propagation methods, like root cuttings and greenwood cuttings, are acutely limited, especially at the beginning of the production. The role of micropropagation can be important at this stage. This was started in the previous Research Institute for Fruitgrowing and Ornamentals in Budapest, which is now known as the NARIC Fruitculture Research Institute.

Poplars were one of the first objectives of in vitro propagation trials (Gautheret 1934, Mathes 1964, Winton 1968, 1970, 1971, Wolter 1968, Chalupa 1974). Cambial tissues were used as basic material in these experiments. First callus improvement, then shoot or root development was induced on the callus surface. Sometimes it occurred without any outside effect. The connection between the regenerated shoots and roots was occasionally unclear. The collected opinions founded the vegetative propagation established from a single bud and different originated callus based plant regeneration (Noh – Minocha 1986).

Whitehead and Giles (1977), Christie (1978), and Ahuja (1983, 1984) micropropagated sterile bud originated plantlets of poplars for the first time. Difficulties of culture establishment and genetically determined differences between the species were pointed out in the presentation of their results. Success of establishment also depends on the age of the mother plants.

Development of micropropagation methods of poplars for commercial purposes was the aim Barocka et al. (1985). Medium optimisation was continued by Chun et al. (1986). Wann et al. (1988) improved the micropropagation method of the tetraploid clone 'Ta – 10' for the substitution of vegetative propagation by grafting. Development of the micropropagation procedure for different poplar species/clones continues today. Coleman – Ernst 1990, Iordan-Costache et al. 1995, Zhang et al. 2000, Noël et al. 2002, Phan et al. 2004, Kang et al. 2009, Thakur et al. 2012 summarised the multiplication procedure of several poplar species that were inoculated or not inoculated with ectomycorrhizal fungus for different experimental purposes.

As the use of micropropagated plantlets became more and more popular, questions of genetic uniformity arose. Rahman – Rajora (2001) used microsatellite analysis to examine a phenotypic homogenous population of *Populus tremuloides* originated from a single mother plant produced by micropropagation. No difference could be detected in 8 out of 10 loci, but differences were observed in the cases of 2 loci. These results offer the possibility of genetic modification during the micropropagation procedure, without any phenotypic change. Genetic uniformity of the traditional methods of propagated plants (grafting, cuttings) was not controlled in the same time.

Breeding work based on in vitro explants started almost parallel with the development of an in vitro mass-propagation procedure for poplars. Protoplast and cell suspension production followed by plant regeneration were the aims of Douglas's (1982) and Ahuja's (1983) research work. Savka et al. (1985) improved the efficacy of breeding methods with early stage embryo isolation and nurturing under in vitro conditions. In the nineties, regeneration trials

for breeding purposes began (Son – Hall 1990, Altman 2003, Confalonieri et al. 2003, Häggman et al. 2007, Yadav et al. 2009, Maheshwari – Kovalchuk 2011) followed with publications on agrobacterium mediated transformation (McCown et al. 1991, Leplé et al. 1992, Song et al. 2006, Nishiguchi et al. 2006).

White poplar selected clones were micropropagated in the laboratory of NARIC Fruitculture RI in collaboration with the NARIC Forest RI. Plant tissue culture methods provide us with new means to speed up the vegetative propagation of selected clones and give us the opportunity to establish new clone experiments and seed orchards (Rédei – Balla 2007).

2 MATERIALS AND METHODS

2.1 Plant material for micropropagation trials

All the clones/cultivars originated from the clone collection of NARIC Forest RI:

Populus alba clones, number: 101. 'H 337' (*P. alba* × *P. grandidentata*),
103. 'H 384' (*P. alba* × *P. grandidentata*),
109. 'H 425-4/1' (*P. alba* × *P. alba*),
111. 'H 425-4' (*P. alba* × *P. alba*),
112. 'K-1' *Populus hupehensis* and
114. 'K-2' *Populus alba* originated from China.

Plant material and growing site for the field evaluation

In the clone trial, clone 101 'H 337' (*P. alba* × *P. grandidentata*), 103 'H 384' (*P. alba* × *P. grandidentata*), 104 'H 325' (*P. alba* × *P. grandidentata*) and 111 'H 425-4' (*P. alba* × *P. alba*) and *Populus* × *canescens* seedlings can be found as controls. The experiment was set up in the spring of 2004 with one-year-old micropropagated plants (for the clones) and one-year-old seedlings (for the control). A randomised block system with three replications was used. The initial spacing was 2.5 × 2.0 m. In every replication, 30 plants were planted. The total experimental area was 0.43 ha. The one-year-old seedlings were produced by the Kiskunsági Forestry Joint Stock Company.

The experiment itself was allocated in subcompartment Kecskemét 40A in the Danube - Tisza interflow region (in central Hungary). The main ecological characteristics of the studied area are: forest steppe climate zone; humidity of the air is less than 50% in July at 2 pm; during the period at the test area the annual precipitation is between 263.8–560.3 mm; hydrology: free draining; genetic soil type: humid sandy soil with very shallow rootable depth. The latitude and longitude coordinates of the experimental area are N 46.883547, E 19.588868.

2.2 Micropropagation

Micropropagation is a useful method for breeders, because a large quantity of uniform plants can be produced in a short time from a single tree or bud.

Steps of the micropropagation method:

1. Establishment of sterile in vitro culture
2. Improvement of multiplication procedure in a growth - room of 22 °C, 1500 Lux light intensity and 16/8 photoperiod.
3. Development of rooting
4. Acclimatization to greenhouse conditions
5. Adaptation under field conditions
6. Plantation

2.3 Field evaluation

Tree classification on the field

Characterization of stem quality, including their health condition, was defined by using the following stem quality classes:

- Class 1* – The stem is straight, cylindrical, healthy and reaching the top of the crown. Crooks are tolerated in one dimension only, up to a bend of less than twice the stem diameter. The lower two-third of the bole is free of live branches.
- Class 2* – The stem is straight and healthy, forks are tolerated, but only if they are in the uppermost third of the tree. Crooks are tolerated in one dimension only, up to less than 4 times the stem diameter.
- Class 3* – The stem is crooked, leaning and more or less damaged. Crooks may reach 6 times the stem diameter in one dimension and minor crookedness in a second dimension is tolerated.
- Class 4* – The stem is very crooked in more than one dimension and heavily damaged. Low branching, forked trees sometimes with broken crown.

The stem quality index was estimated by the arithmetic average of the stem quality classes.

3 DESCRIPTION OF THE METHOD USED AND DEVELOPED FOR MICROPROPAGATION

3.1 Establishment of sterile culture

Three different explants were used for the establishment of a sterile culture of poplar clones:

1. Winter buds were collected in February. After a preliminary disinfection, buds were forced flushing in a thermostat with high relative humidity under artificial light. Shoots developed under controlled conditions were disinfected and planted into growing medium.
This method was found to be successful by disinfection in the case of white poplar. Most of the shoots became sterile, but they stopped growing under in vitro conditions and died after a short time.
2. Shoots developing in springtime, following a careful disinfection, grow quickly.
3. Woody cuttings are not sensitive for disinfection, but often stop further development under in vitro conditions.

Actively growing shoot-tips of a young tree are suitable for culture establishment. In the case of breeding, when the mother plants are about 30 years old or older, the yearly growth is usually only a few millimetres. The early closing buds begin to grow only after a long rejuvenation period.

3.2 Rejuvenation

Rejuvenation can be carried out under 1) in vivo or 2) in vitro conditions.

- 1) In vivo rejuvenation means involving horticultural methods into the procedure of establishment:
 - Rooted cuttings originated from the selected mother tree are grown in a greenhouse (*Figure 1*), with regular plant protection, they are prepared for the establishment (*P. alba* clones number: '109' and '111').
 - Graftings are used as explants ($P \times$ euramericana cv. 'Kopecky').

In vivo rejuvenation is difficult, because any kind of traditional vegetative propagation with adult trees is hard work.



Figure 1. Forced flushing of Leuce-poplars for in vitro establishment

- 2) Rejuvenation in vitro means that the sterile cultures are transferred to fresh medium daily at the beginning, later weekly as far as the fenolic compounds differ into the medium and decrease their inhibition for development (Figure 2). Growth regulators induce flushing at the same time (*P. alba* clones number 101, 103, 112, 114).

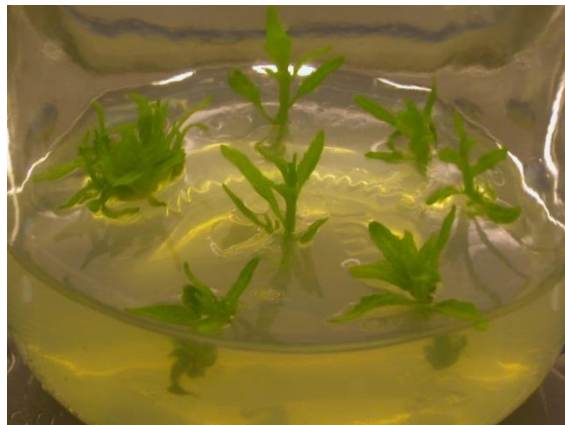


Figure 2. The sterile cultures start to grow

3.3 Multiplication

Experiments for propagation under in vitro condition start when enough sterile culture is prepared. All the cultures are grown in a culture room under controlled conditions: 22 °C, 16/8 day/night photoperiod, 1500 Lux warm white light intensity.

Medium experiments are based on MS (Murashige – Skoog 1962) and WPM (Lloyd – McCown 1980). Poplar shoots are very sensitive to the nitrogen form and concentration as well as for the added growth regulators. They have to be determined clone by clone.

The multiplication rate is typical for the species, clones/cultivars. With poplars, 5–8 new shoots develop monthly. The typical propagation rate can be detected 3 to 5 months following establishment when the cultures were adapted to the in vitro conditions. The developed new shoots isolated from the mother plants are the base of the next propagation phase. The multiplication phase has to be repeated till the required shoot number for rooting is produced. The shoots growing on the propagation medium are elongated and suitable for rooting (Figure 3-4).



Figure 3-4. *Leuce-poplars* in the propagation phase

Vitrification often occurs in white poplar cultures causing a depression of the multiplication rate. Keeping the concentration of major and minor elements as well as growth regulators at a low level can help the cultures avoid these plant-physiological abnormalities. The vitrified cultures have to be eliminated.

3.4 Rooting

Spring time, from February until May, is the best period for root induction.

15–20 mm high, 3-week-old shoots can be rooted in half-strength major and minor elements, reduced sugar containing medium supplemented with a low concentration of auxine.

Rooting rate of *P. alba* clones is about 80–90 percent (Figure 5-6), *P* × *euramericana* cv. 'Kopecky' about 60 percent.

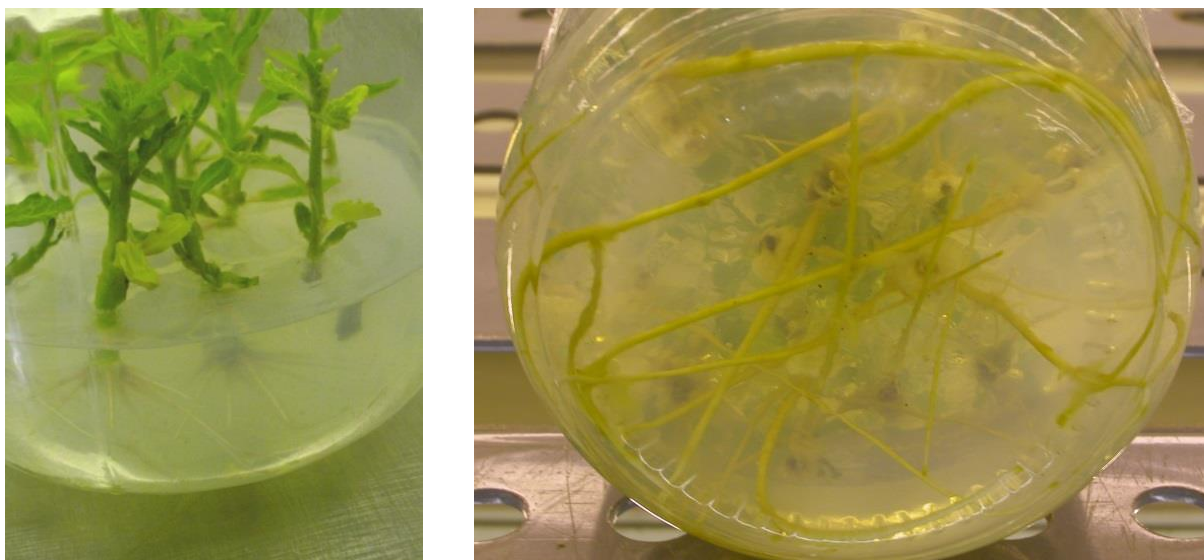


Figure 5-6. Rooted *Leuce-poplars* in vitro

During weeks 3–4, the roots develop and growth of the plantlets achieves the size appropriate for acclimatization (Figure 7).



Figure 7. Rooted poplar plantlets ready for acclimatization

3.5 Acclimatization

Acclimatization means the period (4–6 weeks) where the plantlets – developed under sterile conditions – are adapted to semi-sterile greenhouse conditions. This is one of the most difficult parts of the micropropagation procedure. Spring is the best time for acclimatization, because days grow longer and the greenhouse temperature can be kept between 15–30 °C.

The quality of the substrate has to be chosen to fit the requirement of the plant species. With trees, usually fibre, – reach substrate with high water capacity and a pH of about 6–6.5 can be used. Nutrition can be contained in the substrate or can be added together with the water supply.

Following the transplantation of plantlets into the substrate, a relative humidity of 90-95% has to be ensured for them until the stomas begin to work and the development of plant skin is finished (about 10 days). After then the relative humidity can be reduced to the normal greenhouse level. Protection against direct sunlight is also important during the whole procedure.

Attention must be paid to plant protection, mainly against fungal infection. By the end of the acclimatization, the plantlets grow up to 15–20 cm (Figure 8), which is a good size for transplantation under shaded and irrigated nursery conditions (Figure 9).



Figure 8. Acclimatization of different poplar clones in the greenhouse



Figure 9. Growth of the white poplars in the nursery garden

Plantlets acclimatized in the mentioned period in the first growing season grow to the size fit for grafting in August–September in the case of rootstocks, or for reforestation in October–November in case of forest-trees (Figure 10, 11, 12).



Figure 10. Micropropagated H-325 Leuce poplar clone in the nursery garden



Figure 11. Growth of the H-384 clone in the nursery



Figure 12. H-337 white poplar clone in the nursery

Hot summer and dark winter periods are not fit for acclimatization. Tree plantlets, acclimatized in autumn are difficult to keep during the winter period, so it is not suggested.

4 RESULTS

The evaluations have proved that the clones *P. alba* × *P. grandidentata* H-337 and H-384 are the most suitable for poplar growing under unfavourable ecological factors, while the clone *P. alba* × *P. alba* H 425-4 could be considered as an alternative for wood production. The abovementioned promising clones may also be planted in roadside plantations and parks because of their decorative value.

Table 1 shows the most important stand structure parameters of the Leuce-poplar clones at the age of 8 (Kecskemét 40A). Based on the data concerning the growth in height, clones H-337 and H-384 provided the best results. They surpassed the control by 51% and 22% respectively. Regarding the growth in DBH (diameter at breast height) the abovementioned clones surpassed the control by 30% and 25%; the tendency was also the same with mean tree volume values. The effect of differences in DBH on the mean tree volumes seems to be very considerable (an additional 102% and 49% for the above-mentioned clones). Where the stem quality index is concerned, the succession from best to worst is: H-337, H-384, H 425-4, H-325 and the control. According to the significance test at $P = 5\%$ level, significant differences

were found in height ($SD_{5\%} = 2.07$ m), in DBH ($SD_{5\%} = 2.11$ cm) and in the mean tree volume values ($SD_{5\%} = 21.2$ dm³) (Table 2).

Table 1. Some yield data and stem qualification of Leuce-poplar clones at age of 8 years (Kecskemét 40A, 2011)

Name of clone	Mean height		Mean diameter at breast height		Mean tree volume		Stem quality index (1–4)
	(m)	%	(cm)	%	(dm ³)	%	
H-325	8.05	111	7.40	102	25.8	102	2.14
H-337	10.95b	151	9.44b	130	51.3b	202	1.33b
H-384	8.84	122	9.08	125	37.9	149	1.61b
H 425-4	7.66	106	7.82	108	26.6	105	1.91
Control	7.24a	100	7.26a	100	25.3a	100	2.28a
SD_{5%}	2.07		2.11		21.2		0.52

Between the values marked by different letters significant differences were found at $P=5\%$ level.

Table 2. ANOVA values according to the investigated parameters.

Height				
	SS	DF	MS	F
Total	50.93	20		
Replication	3.43	2		
Treatment	30.82	6	5.1366	
Error	16.68	12	1.39	3.69
$SD_{5\%} = 2.07$				
DBH (diameter at breast height)				
	SS	DF	MS	F
Total	32.21	20		
Replication	2.32	2		
Treatment	12.48	6	2.08	
Error	17.41	12	1.4308	1.43
$SD_{5\%} = 2.11$				
Mean tree volume				
	SS	DF	MS	F
Total	0.003774	20		
Replication	0.000307	2		
Treatment	0.001761	6	0.00029	
Error	0.001706	12	0.000142167	2.06
$SD_{5\%} = 21.2$				
Stem quality				
	SS	DF	MS	F
Total	3.270000	20		
Replication	0.200000	2		
Treatment	1.890000	6	0.3150	
Error	1.180000	12	0.0983	3.20
$SD_{5\%} = 0.52$				

$P < 0.05$

The collected data were analysed using the STATISTICA 8.0 (data analysis software system -StatSoft, Inc. 2008) programme including correlations and regression analysis.

The annual ring evaluation analyses show that selection surplus can also be detected under marginal site conditions. According to the average annual ring width (growth in diameter at breast height), clone H-384 surpassed the control by 52.7% while clone H-337 surpassed it by 46.2% (Figure 13).

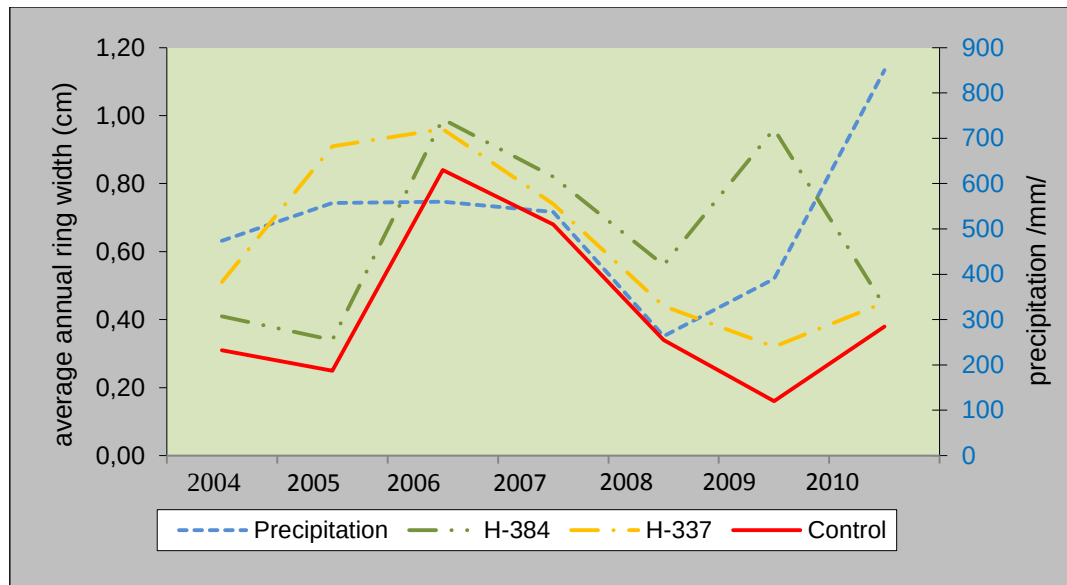


Figure 13. Annual ring analysis of the 2 most promising *Leuce-poplar* clones at the age of 7 (Kecskemét 40A, 2010)

We have done discriminant analysis considering the DBH (diameter at breast height), height, rate of growth, stem quality, and health condition as quantitative variables – according to the different clones – as observation groups. Figure 14 shows the ordination diagram based on canonical discriminant functions of the variety comparison clone trial.

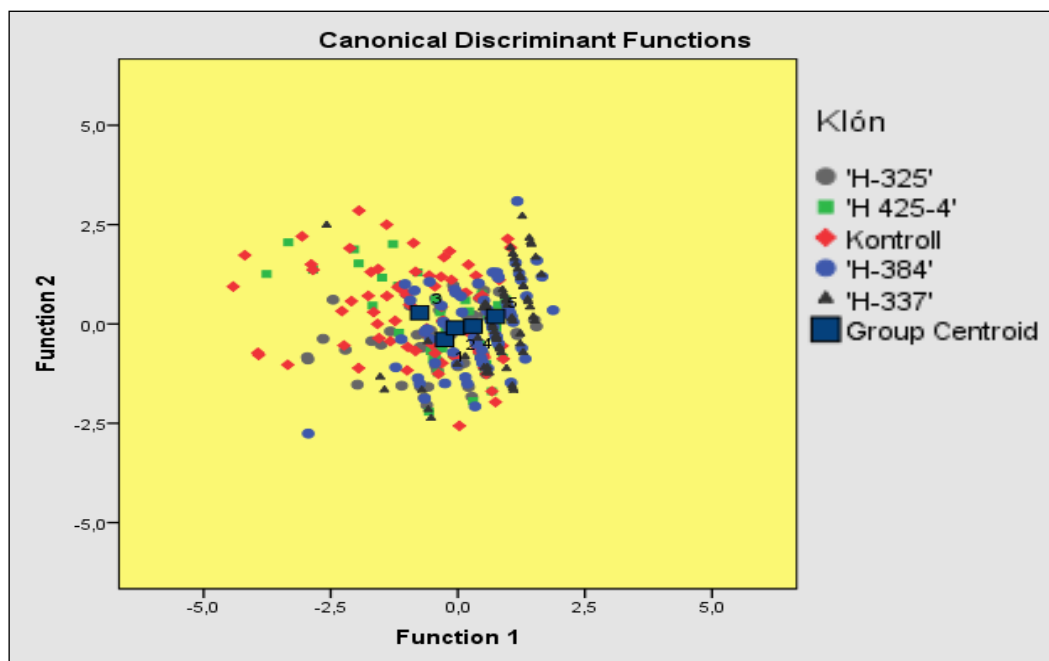


Figure 14. The common ordination diagram of the 5 groups (clones) on the basis of the investigated characteristics

In the course of the discriminant analysis it can be said that if the number of groups are more than 2 – as in this case – the discrimination of cases (variables) belonging to the single groups (clones) can be done with the help of so-called canonical variables.

Figure 14 indicates – though not clearly – that the groups separate from each other considering the first function (horizontal axis). The figure also shows that the control (commercial) white poplar specimens show the highest deviation according to the investigated characteristics compared to the other clones.

5 DISCUSSION

The H-384 and H-337 clones presented the most homogeneous distribution. These results also prove the excellent stem, form, yield properties, health condition, and the outstanding rate of growth of these 2 promising *Leuce-poplar* clones (Keserű 2013).

In spite all of the efforts to reduce the cost of the micropropagation, using micropropagated plants for reforestation seems to exorbitantly expensive given present economic circumstances. Today a micropropagated plant costs 2 EUR, while the price of a commercial seedling is much lower (cca. 0.1 EUR). In Hungary and also in other countries (Haapala et al. 2004) mother plantations are established from micropropagated plants and from there trees for forestation are propagated year by year using conventional vegetative propagation methods, usually by cuttings (Lubrano 1992, Confalonieri et al. 2003).

Among the forest species, poplars belong to a well-known group and can be used as a model tree for other species (Taylor 2002). The production of clones that grow quickly in shorter periods of time, are adaptable to different sites like poor, polluted soil or drought conditions, and can yield more wood is currently ongoing through the application of biotechnological methods that are based on micropropagation (Lubrano 1992, Giri et al. 2004, Doty 2008, Chen – Polle 2010). Experiments on the production of genetically modified resistant clones against fungal and lepidopteran infections using *Agrobacterium* mediated transformation methods are improving (Zhang et al. 2005). The important role of poplars among the energy tree species must be stressed (Polle – Douglas 2010, Sanningrahi et al. 2010).

6 CONCLUSIONS

The early evaluation demonstrated in the paper showed that the micropropagated clones H-337 and H-384 seem to be suitable for poplar growing under unfavourable site conditions, while clone H 425-4 could be considered as an alternative for wood production. The experiments have also demonstrated that micropropagated plants can be successfully transplanted into soil, hardened, and grown in the field. Micropropagated trees have been exhibiting normal growth and appearance since they were planted. Hungary has a great deal of experience growing white poplars. A selected clone that has a really high growth capacity, as is demonstrated in the present publication, should be used for reforestation or ornamental purposes despite the higher cost.

ACKNOWLEDGEMENTS: SUPPORT OF THE MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT (PROJECT NUMBER: 9 – FVM/K00557/1/2004) IS DEEPLY ACKNOWLEDGED.

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Multi-Frequency Electrical Impedance Measurement on a Wooden Disc Sample

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Abstract – Non-invasive measurement techniques are widely used for the investigation of living tree and wood material. Principles and methods from other fields of science form the basis of these techniques. Electrical impedance measurement, which is adopted mainly from applied geophysics, plays an important role in the investigation of living trees and wood material. Unlike other measurement techniques that provide mainly physical information, electrical impedance measurement is able to gather either physical or chemical information. There are several published accounts of the use of Electrical Impedance Tomography mainly for examining internal decay, estimation of sapwood, and heartwood width. The advantages of the method are its low costs and rapid execution; however, low spatial resolution is still one of the most glaring limits of the method. Use of a new, self-developed, high resolution measurement system provides new opportunities to gain better insights into the characterisation of wood material. As well as high accuracy and resolution, the measurement system is able to operate in a wide frequency range. This expands the examined parameters making it possible to extend the gained information and develop new methods for the investigation of living trees and wood material. The aim of our publication is to introduce some experimental results in the investigation of spatial resolution our system measured on a wooden disc.

multi-frequency Electrical Impedance Measurement / spatial resolution / wood disk

Kivonat – Fakorongok multi-frekvenciás elektromos impedancia mérése. Az élőfák és faanyagok vizsgálatához különböző mérés technikai eljárásokat alkalmaznak. Ezek alapját elsősorban más tudományterületekről hozott elvek és módszerek képezik. Az elektromos ellenállás (impedancia) mérés, melyet a geofizikai gyakorlatból adoptáltak, ezek között nem kevésbé fontos szerepet tölt be, ugyanis míg a mérési módszerek többsége szerkezeti, fizikai információt szolgáltat, addig az elektromos módszerek a fizikai mellett kémiai információszerezésre is alkalmasak. Az Elektromos Impedancia Tomográfiát több ízben publikálták élőfák esetében üregek, illetve szíjács és geszt vastagság becslésre. Bár a mérési eljárás előnyei közé sorolható a relatív alacsony költségigény és gyorsaság, összevetve azonban más képalkotó eljárásokkal, a kisebb térbeli felbontás szab határt az egyes alkalmazások elterjedésének. Egy új, saját fejlesztésű nagy felbontóképességű mérőeszköz alkalmazása új lehetőségeket nyújthat a felbontás javításában és a faanyag jellemzésében. A mérőeszközre jellemző, hogy a nagy pontosságú és felbontóképességű méréseket igen széles

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frekvencia-tartományban képes kivitelezni. Ez egy új dimenziót nyit meg az élőfa és faanyag vizsgálat módszertanában, mely egy új mérési eljárás fejlesztését alapozhatja meg. Jelenlegi publikációnk célja mérőeszközünk térbeli felbontásának kísérleti ellenőrzése faanyagon.

multi-frekvenciás elektromos impedancia mérés / térbeli felbontás / fakorong

INTRODUCTION

Non-destructive measurement techniques play a crucial role in the evaluation of living trees. These methods are developed mainly from imaging techniques used in applied geophysics (Szalai et al. 2002) and medical diagnostics. Imaging techniques from the adaptation of seismic methods are the most improved. (Divos et al. 1994) These measurements are made by using acoustic waves. The result of acoustic tomography is generally an approximation of the spatial distribution of wave velocity in the investigated tree. There are several other non-destructive imaging methods published mostly for the detection of decay in trees. (Divos et al. 1991, Nicolotti et al. 2003, Bucur 2003) These investigation methods are X-ray, gamma ray, computer tomography, nuclear magnetic imaging, thermal techniques, ground penetrating radar, microwave methods and electrical tomography.

Electrical tomography methods are used in geophysical sub-surface explorations by performing on-surface measurements. As an early woodmaterial investigation method, electrical resistivity measurements were published in (Shortle et al. 1997). The researchers (Shortle et al. 1997) examined the spatial distribution of electrical resistance in a wooden disc. The authors performed the published measurements on samples taken from the wooden disc. Later, other authors published electrical measurement methods applied to several goals, for example finding discoloured wood, red-heart in beech (Weihs, 2001), detection of decay in trees (Dubbel et al. 1999; Rust et al. 2007, Brazee et al. 2011); estimation of sapwood and heartwood width (Bieker et al. 2010.); variation of electrolytes in *Quercus robur* (Bieker et al. 2010.). For these purposes, Electrical Resistance Tomography (ERT) as a non-invasive measurement technique was used. In the case of these investigations, the measurements and evaluations were performed with single frequency measurements.

In multi-frequency Electrical Impedance Tomography, we create an electrical field in the segment of a living tree that is to be investigated. This field depends on spatial distribution of admittance (or impedance), which is characteristic for the tissue of the tree. Admittance (reciprocal of impedance) is a physical quantity, which measures how easily an investigated material allows the injected current to flow through it. The electrical field can be characterised by the measuring of electrical potentials on the boundary of domain. Through data collection and evaluation, the spatial distribution of admittance (or impedance) can be approximated. The speciality of mfEIT is the injected current, which is a monochromatic sine wave. Thus the measured material can be characterised in time and frequency domain. (Woo et al. 2007) Testing in time domain includes the EIT in classical meaning. By adding the measurements in frequency domain, we can measure the transfer function of the object. Thus we gain a more extended evaluation method for the investigation of living trees and wood materials that includes the measurement of spatial distribution of admittance (or impedance) depending on the frequency. The investigated structure responds dissimilarly in every frequency point.

The aim of mfEIT is to locate several types of anomalies in living trees in the best spatial resolution possible. To achieve this, equipment with high resolution and an appropriate electrode configuration is essential. A publication of Beck - Williams (1996) compares the advantages and disadvantages of several techniques applied in industrial tomography.

According to their statements (1996), EIT is a relatively cheap, quick, suitable, and simple imaging technique; but there is a need for research in the refinement of spatial resolution which is relatively small when compared to other techniques. The spatial resolution of EIT is determined as the ratio of diameter of the smallest object that can be located and the average diameter of material in which the measurements are made. According to Beck - Williams (1996), the EIT spatial resolution is about 10%, the worst imaging technique in industrial tomography. A spatial resolution of 10% means that EIT as an imaging method has the capability to localize a 20 mm object (anomaly) in a 200 mm diameter sample.

MATERIALS AND METHODS

The measurement system consists of a high precision generator and a measuring board. The generator, which can be operated as a current or voltage generator, provides a low-distortion monochromatic sine wave for the excitation of a measured object in a large frequency range, between 20 mHz and 90 kHz. The measurement board is in general a data acquisition system that measures the real and imaginary part of complex voltages with 48 bit resolution. Amplitude measurement accuracy is 1 ppm and phase measurement accuracy is 0.01°. The system can execute spectral (Fourier spectrum of signal), spectroscopic, and tomographic measurements. With wobbler function it is possible to record impedance spectrums and transfer functions. Tomographic measurements are realized with parallel measurements using 8 channels. By adding a multiplexer board, it is possible to extend the number of channels up to 64. Our primary aim is to develop a modern tomographic method, which is called multi-frequency Electrical Impedance Tomography (mfEIT), for the investigation of living trees using a measurement system with high accuracy and resolution. In our paper we demonstrate the results of the experiments dedicated to spatial resolution determination in wood material.

In order to verify the suitability of our measurement system for mfEIT measurement in living trees and wood material, we performed an experiment for spatial resolution determination. We measured wood samples several times – first without an anomaly and later with an artificial anomaly. A fresh (green) slice of hornbeam (*Carpinus betulus*) was chosen for the experiment. The height of the disc was 11 cm; the average diameter was 20 cm. The measurement setup can be seen in *Figure 1*.

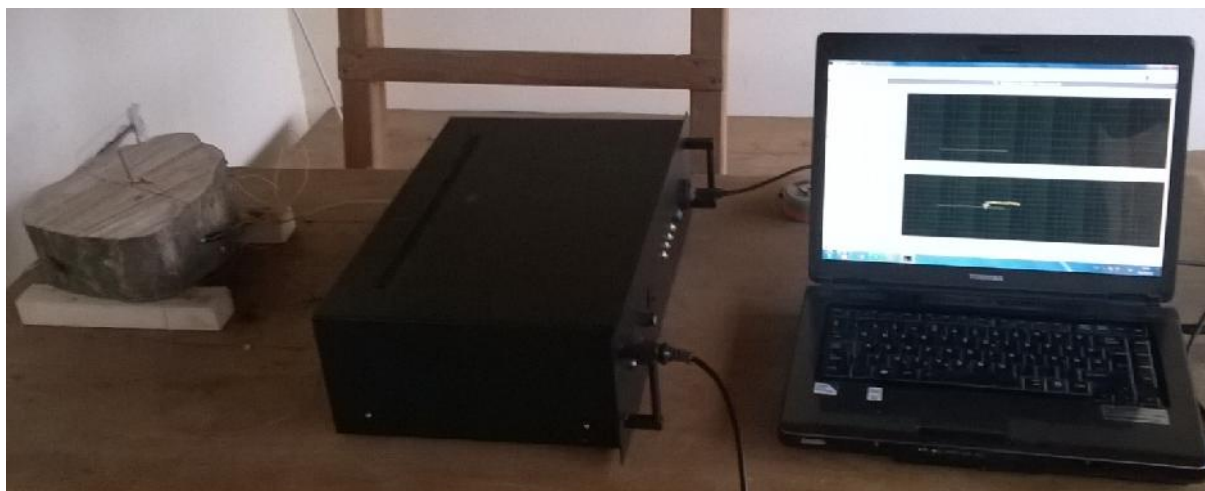


Figure 1. Measurement layout for experiments in hornbeam

In the first case the log was measured without anomalies. These recordings were used as background information in the evaluation. Then an anomaly – a small hole – shown in *Figure 2*, was made in the sample. Later, additional holes were drilled in the hornbeam disk. The holes were placed on the line between the source electrodes; the distance of the negative pole of generator and the centre of the bore was 140 mm. The diameter of the first hole was 8 mm (4% of the sample diameter); this was increased to 10 mm (5%), 12 mm (6%), 16 mm (8%), 20 mm (10%).



Figure 2. 8 mm diameter bore, drilled into slice of hornbeam for experimental measurements

In this study we applied only 4 electrodes in addition to a ground electrode which was located at the centre of the disk. In the case of in-situ measurements, we will place the ground electrode in the soil, near the tree. The applied electrode configuration is shown in *Figure 3*.

The electrode configuration shown in *Figure 3* is called a null array in geophysical practice (Szalai et al. 2002), which indicates some types of anomalies very sharply. If the sought anomaly doesn't exist, the measured values are minimal corresponding to the inhomogeneity of measured material. When an anomaly can be found in the investigated material, the measured potential values respond with very sharp changes. *Figure 3* shows that the current generator was connected to the source electrodes (1 and 2) and the potentials were measured on electrodes 3 and 4. The lines, linking the source (1 and 2) and measuring (3 and 4) electrodes were perpendicular. That results in the appropriate sensitivity that is characteristic for null arrays.

We measured the complex voltages on the source and measuring electrodes by applying a current generator. For calculations we applied the complex voltage measured on source for reference voltage. The measurements were done with swept sine in a range of 0.1 Hz – 90 kHz, in 100 frequency points. The voltages, measured on electrodes 3 and 4, are dissimilar to the reference voltages in every frequency point. This difference, caused by the measured material, can be characterized by calculating the attenuation of amplitudes and phase difference. (*Figure 4*).

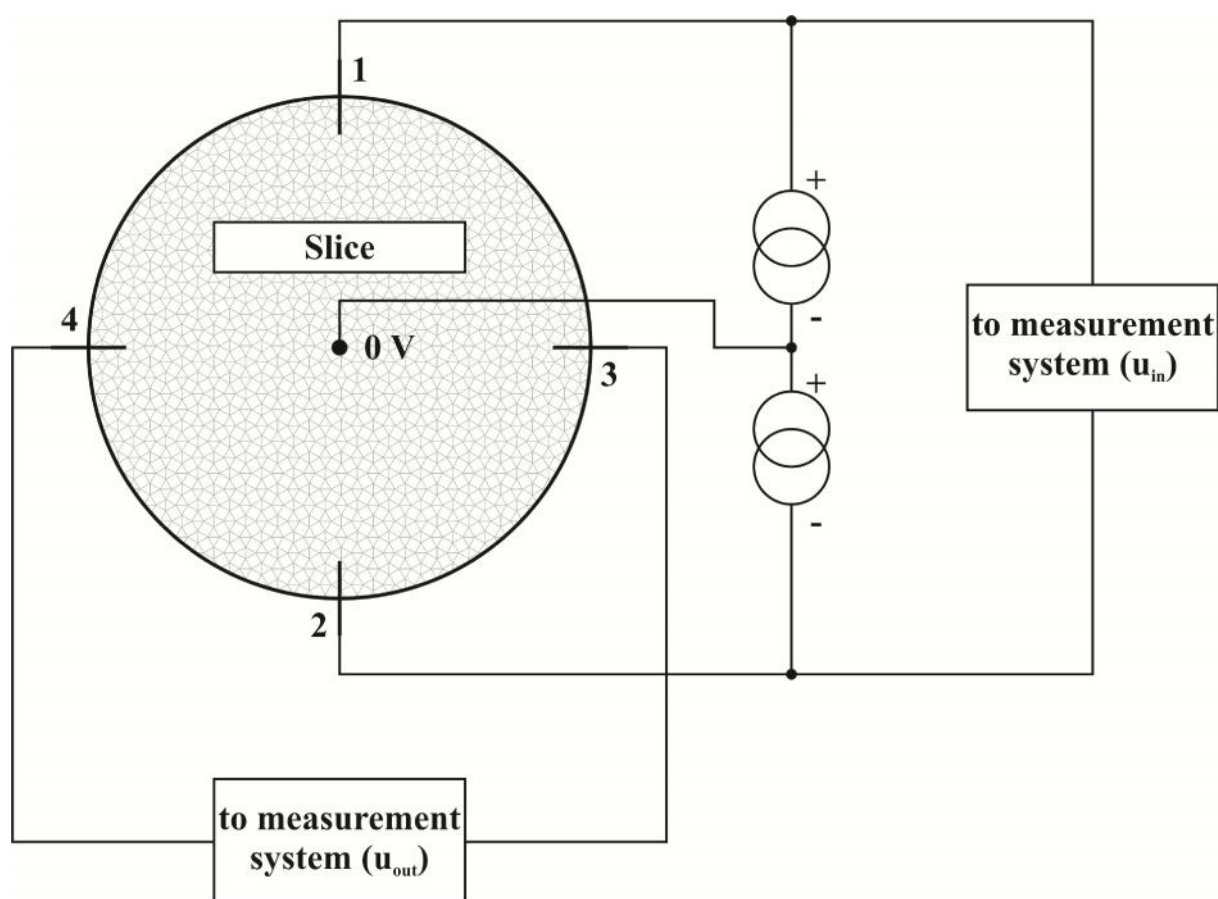


Figure 3. The applied electrode configuration

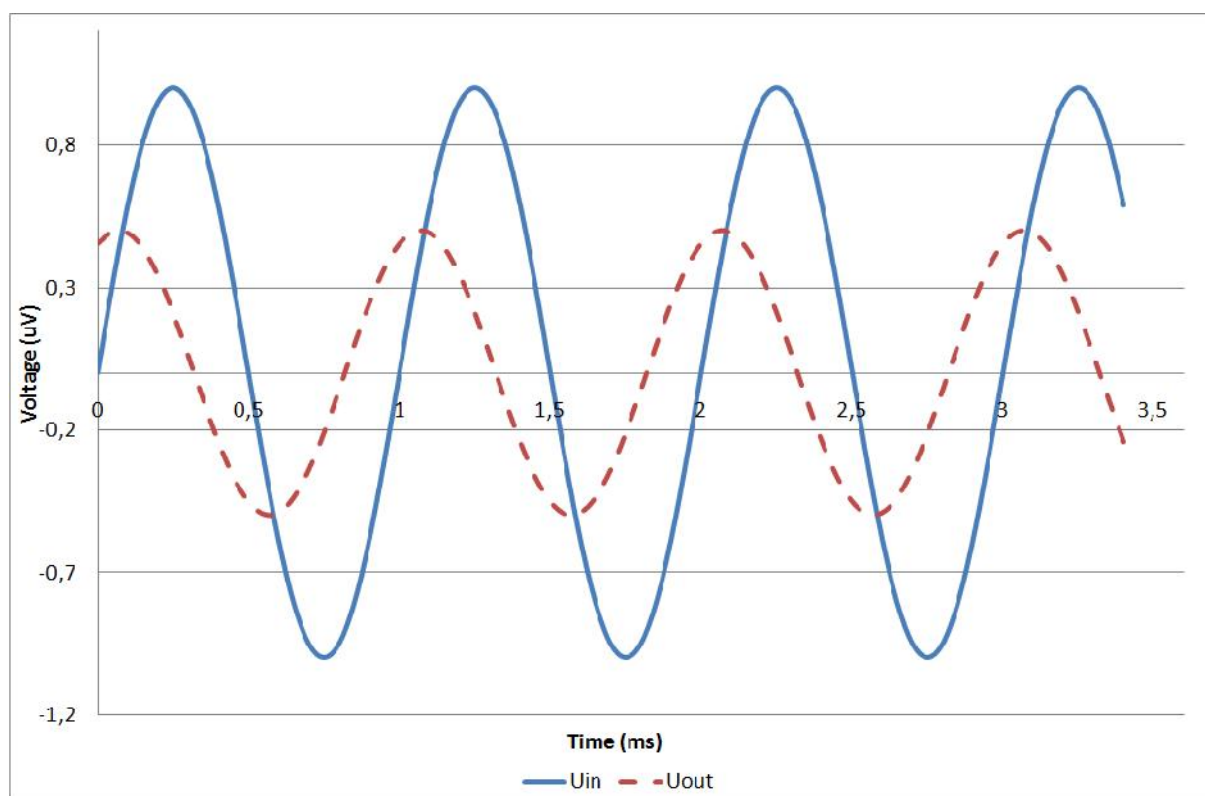


Figure 4. Illustration of the difference between the measured and reference voltages measured in linear material

The collected data is characteristic for the transfer function of the tested material. Thus the evaluation of collected data is possible in this way:

$$A = -20 \cdot \lg \frac{|u_{out}|}{|u_{in}|} \quad (1)$$

$$\varphi = \varphi_{in} - \varphi_{out} \quad (2)$$

where

A is the attenuation of signal in measured material (dB)

$|u_{out}|$ is the magnitude of voltage measured on electrodes 3 and 4

$|u_{in}|$ is the magnitude of voltage measured on source electrodes

φ is the phase difference

φ_{out} is the phase of voltage measured on electrodes 3 and 4 (°)

φ_{in} is the phase of voltage measured on source electrodes (°)

We assumed homogeneous electrical properties in the radial – tangential plane, although in reality, according to our experimental data, there is about a 10% relative difference between radial and tangential directions. At this stage we tried to ignore this effect, but in the future we will need to take this into consideration.

RESULTS

The first measurements were made in a log of hornbeam without artificial defect. The aim of these experiments was to gain information of the electrical stability of the measurement setup. These measurements were used as background data as well. For the characterisation of stability of the measurement set-up, single frequency recordings were made with 1 Hz, 5 s averaging time in 60 s lengths. Coefficients of variation (CV) were calculated. The CV values in general were better than 0,5% (at 1 Hz). Thereafter the transfer functions were measured. In every experimental case the transfer function was recorded 3 times. From these the average CV values were calculated, which were better than 0,05% for magnitude and better than 10% for phase (calculated on the total frequency range).

The measured transfer functions for all experimental cases are shown in *Figure 5*.

Figure 5 shows the attenuation and phase versus frequency – called a Bode diagram. Both of these characteristics are specific for the investigated material. Thus, for the evaluation of the measurements, both of the curves have to be taken into consideration. The attenuation is represented in dB unit and the phase in degree. According to *Figure 5*, the attenuation curves have the same character. At low frequency, they have maximal values and they decrease monotonically. The shape of attenuation curves is specific for the material properties. The difference between maximum and minimum attenuation values is 3.29 dB in the case of measurement without bore. The phase diagrams are characteristic for the wood material. At low frequency the phase starts on the minimum values and then it increases until 100 Hz before it decreases and increases again. The dynamic range of phase alternation is 9.89°.

Figure 5 clearly shows the differences between experimental cases. The hole drilled into the log in physical aspect is equivalent to placing a non-conductive media into a conductive. This coarse intervention should result in significant change in the measured attenuation. This can be seen in *Figure 5* as well. The smallest attenuation values were measured without an anomaly. Then with the addition of non-conductive media, the attenuation values increase

consequently. The experimental cases can easily be distinguished in *Figure 5*. The correlation of attenuation curves and the size of the bore can be clearly seen. The phase values indicate the anomaly clearly as well. By increasing the diameter of non-conductive media at low frequency, the phase is decreasing. Between 1 Hz and 4 kHz the phase curves have nearly the same values. Thus in this frequency range, the phase measurement isn't capable of distinguishing the diameter of an anomaly. At higher frequencies the phase curves become separated and they indicate the diameter of non-conducting media. Contrary to the change at low frequencies, at a higher frequency range according to the increasing diameter of an anomaly, the phase curves shift to higher values. It is evident that phase measurement provides new information about the investigation of trees and wood material. If an anomaly exists in investigated media, the measured phase curves will characterise the anomaly. Thus by combining the tomographic measurement with the recording of the transfer function of investigated tree or wood material, a characterisation of an existing anomaly can be gained.

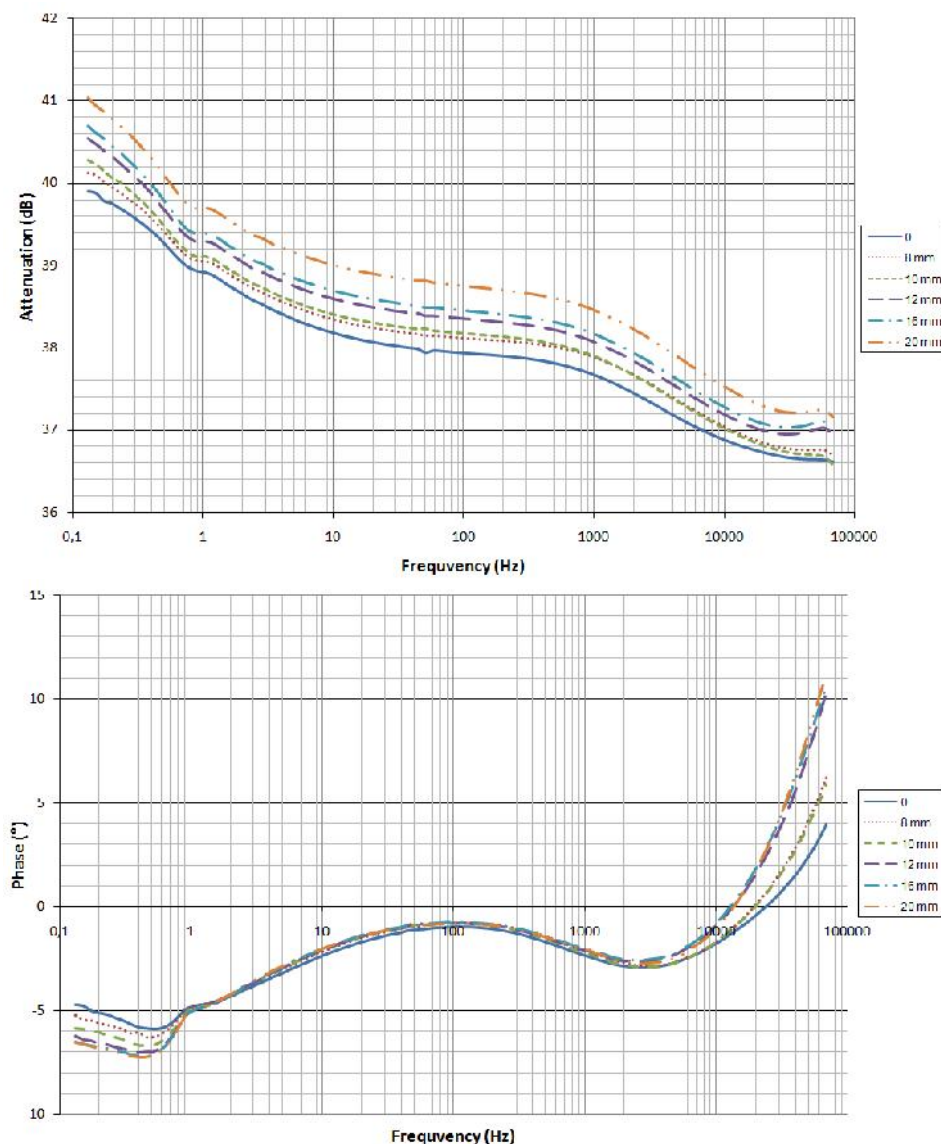


Figure 5. Transfer functions measured in hornbeam log without (0) and with non-conducting media of several diameters

The obtained results clearly show that the multi-frequency approach offers more extended information about the investigated wood material than the traditional EIT, or ERT methods. Evaluating the attenuation of measured signals represents a possibility to draw conclusions about electrical properties, size and shape of an investigated anomaly. The published experiment shows that the attenuation curves (*Figure 5*) – according to the results performed without a bore – are in correlation with the diameter of bores: with the increasing diameter of a bore, the difference of actual and 0 curves is increasing. The phase curves (*Figure 5*) represent the extended information, which is a result of multi-frequency investigation. The 0 curve is characteristic for wood material. With the addition of an anomaly, a non-conductive media (bore), the shape of phase curves became distorted. This alteration is in correlation with material properties of the added anomaly. The phase curves can be examined in low, middle, and high frequency intervals because the response of curves is different in these intervals. This is an important opportunity for enhancing the selectivity of measurement.

Summarising the results: using the multi-frequency approach offers a possibility to develop a mfEIT tool for tree evaluation with better – at least 4% – spatial resolution compared to the 10% reported in the literature, Beck (1996).

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Guide for Authors

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Contents and Abstracts of the Bulletin of Forest Science

Bulletin of Forest Science (Erdészettudományi Közlemények) is a new journal supported by the Hungarian Forest Research Institute and by the Faculty of Forestry of the University of West Hungary. The papers are in Hungarian, with English summaries. The recent issue (Vol. 5, 2015) contains the following papers (with page numbers). The full papers can be found and downloaded in *pdf* format from the journal's webpage (www.erdtudkoz.hu).

Vol. 5, 2015

Tamás MAJOR and Viktória CSANÁDY:

Combined FEM-SPH simulation method for the modelling of the interaction of tillage tools and the soil ... 7-19

Abstract – Earlier cultivation and tillage tools were designed on the basis of practical experience; testing and records of fundamental theoretical analysis of these tools is largely lacking. Today, the release of new equipment is always preceded by several years of laboratory tests and field experiments. By modelling the interaction between the soil and tillage tools, the time and costs of the development process can be significantly decreased. Thanks to the recent rapid advancement of high performance computers and numerical methods, it is possible to complete these model calculations more effectively now. With the help of a combined FEM/SPH simulation – in this case utilizing two different driving speeds of a tractor – we determined the magnitude of horizontal force acting upon the rotating tool as a function of time. Using the functions fitted to the simulation results, the authors have calculated the average and the maximum values of the horizontal force. Moreover, normal stress distribution in the soil has also been determined.

János RUMPF, Attila László HORVÁTH and Katalin SZAKÁLOSNE MÁTYÁS

Tree utilization price revenue categories and quality classification of some trees and forest stands ... 21-41

Abstract – The „Simplified grading selection method” (Szász és Burján 1975) developed decades ago by the Forest Research Institute (FRI) and founded upon an earlier range of selection methods and evaluations of the diameter at breast height for logging became obsolete and necessitated updating and modernization. This was adjusted to the prevailing current logging data in order to make valuable original data useful and useable for the design and evaluation of current logging practices. We developed a simple solution for this: the so-called two-step proportioning method. However, the use of FRI tables requires the need to know the quality of tree stands as well. For this purpose, we utilized the trunk rating system in the Forest Planning Guide, making some adjustments to suit the task at hand. The resulting selection structure multiplied by current average prices determined average revenue. Our

method proved suitable for different levels of aggregation including national, forest company, and forest subcompartment levels. The process can be considered as a „sales-centric quality classification” of stands. To calculate the overhead cost of logging operations, we utilized the suitable portion of the developed cutting organizational plan (Szasz, 1979), and named the modified version the "Sopron series analysis method".

Ferenc FACSKÓ

The transparency of forestry companies in 2014 ... 43-53

Abstract – The following report summarizes the results of a study which examined the level of transparency among state-owned forestry companies. Overall, it was discovered that value of forestry companies are around country average. The information content of forest company websites can be assigned to two categories: a wealth of information or very little information. It can be said that companies that do not meet their legal requirements are also more likely to provide little information or information of poor quality on their websites.

István BACH, Norbert FRANK, Beáta PINTÉR and Sándor BORDÁCS

Changes in the production of reproductive material in forest management from the years 1982-2014 (Quo vadis forest reproductive material production?) ... 55-69

Abstract – Over the past decades, the forest reproductive material sector has changed significantly in Hungary. Societal, economic, and political developments have decisively influenced forest management, the results of which are reflected in the statistical indicators of the production of forest reproductive material. Analyses of statistical data point out relevant trends and changes, especially in ownership structure, total number of nurseries, mean size of nursery area, and proportion of tree species produced. In the early 1990s the formerly dominant state-owned nurseries were replaced by privately-owned ones, and large or medium size nurseries were replaced by small or even micro size farms. Simultaneously, the mean area of nurseries decreased drastically for about 10 years. As a consequence of slight reduction in total number of nurseries, the mean area, as well as the total volume of reproductive material produced by each nursery, has increased slightly since the 1990s. In general, relevant modifications in forestry policy, such as the increasing importance of close-to-nature forestry, modified preferences in the use of non-autochthonous tree species, and essential changes in afforestation programs have significantly affected the production structure of the forest nursery sector as well. For example, the production volume of reproductive material of conifers is far less than it was in the 1990s, but the production volume of scattered broadleaves is far greater than it was in the 1990s. Further variations in the structure of tree species can be expected due to the varying needs of climate change.

Bence BÁRDOS, László NAHÓCZKI, Dénes MOLNÁR, Norbert FRANK, Zoltán KÖVESKUTI and Ádám FOLCZ

Investigation of epicormic shoot growth of sessile oak in shelterwood cutting stands ... 71-83

Abstract – This paper addresses the epicormic shoot growth of sessile oak (*Quercus petraea*) in natural regeneration stands which can have a strong effect on potential trunk quality at harvesting; the deterioration of trunk quality can cause significant economic losses for forest managers. During the course of our research, we investigated the epicormic shoot growth of sessile oaks in shelterwood cuttings in different ecological environments. We examined 487 specimens on 10 plots in 3 different Hungarian regions. The collected data were analyzed in relation to site conditions and silvicultural interventions. Our results show that epicormic shoot growth intensity is influenced by crown size and changes in environmental parameters.

Accordingly, it is recommendable to make more intensive cuttings in young stands in order to stimulate trees to grow larger crowns. Faster and more careful final cuts are also suggested. By using these principles, trunk quality deterioration caused by epicormic shoot growth can be mitigated.

Árpád SZALACSI, Szilvia VERES and Gergely KIRÁLY

Gap cutting and its effects on the understory vegetation in the pedunculate oak-hornbeam forests of Szatmár-Bereg Plain (NE Hungary) ... 85-99

Abstract – Lowland oak-hornbeam forests are one of the important sites of quality oak wood production. Consequently, this habitat has become a major conflict point between forest management and nature conservation in Hungary over the past 20 years. In order to develop a regeneration method based on natural processes, we tested the impacts of gap regeneration cuttings in three compartments in the Szatmár-Bereg Plain (NE Hungary). During the regeneration process, we found an insignificant number of invasive weeds in the gaps. Forest herbs were represented nearly uniformly in the gap parts variably exposed to the sun; the cover of native weed species was significantly higher in the central part of the gaps. Mixed tree species (especially hornbeam) have shown great vitality in the gaps. Thus, in order to ensure the natural regeneration of the pedunculate oak, protection against game damage and proper management are indispensable. Based on our observations, a recommended minimal starting gap size in lowland hornbeam-oak forests is 0.15 hectares. After they are created, further expansion of the gaps is necessary after 4-5 years. The final restoration subdivision (approx. 2 ha) can be reached in 2 steps within a span of 8-10 years. The forests created in this way are mixed and are of an appropriate vertical and horizontal structure.

Tivadar BALTAZÁR, Ildikó VARGA and Miloš PEJCHAL

The impact of the European mistletoe (*Viscum album* L.) on woody host-plants: a study of the relationship between infection intensity and tree vitality ... 101-118

Abstract – Our research aims were to examine the relationship between infection intensity of European mistletoe (*Viscum album*) and the physiological and biomechanical vitality of the potential host species. For this study 3039 individuals of nine host species (*Acer campestre*, *A. platanoides*, *A. pseudoplatanus*, *Crataegus monogyna*, *C. pedicellata*, *Juglans nigra*, *Robinia pseudoacacia*, *Tilia cordata* and *T. platyphyllos*) were examined; of these 1424 specimens were infected. The host trees are situated in the Castle Park in Lednice, Czech Republic. Based on our results, it can be concluded that there is a strong relationship between these three factors. The weakest relationship was observed in the case of field maple (12-17%) and the strongest relationship was in the case of the black walnut (32-39%). In spite of our findings, the exact role of tree vitality influencing mistletoe infection remains unclear.

Szabolcs SZANYI, Levente SZŐCS and Zoltán VARGA

The zoogeographical and ecological characteristics of Macroheterocera fauna of the Bockerek forest reserve ... 119-128

Abstract – The Bereg Lowland is the most humid and coolest part of the Great Hungarian Plain, and is also the richest in natural or close-to-natural forests. Among them, the Bockerek forest (between Tákos and Vámosatya) is one of the most important. It is a nature conservation area and a large portion is a forest reserve. Its condition needs to be continually monitored; thus, the Forestry Research Institute has been monitoring the forest with light traps for a considerable time. From this material we analysed the faunal list of the nocturnal macro-moths. Since we only used a taxonomic list of species from a 9-year period without

frequency data, we only surveyed the composition according to faunal types and ecological faunal components with special regard to significant pest species in forestry.

József VARJU and Ferenc JÁNOSKA

Woody nutrient preferences and habitat use of the Eurasian beaver (*Castor fiber* Linnaeus, 1758) at the Moson Danube ... 129-144

Abstract – Beavers (*Castor fiber*) inhabiting the Moson Danube region presently cause considerable damage to the forestry sector. In our study we discuss forest areas affected by the activities of the beavers. We marked out plots in economically significant forest cultures in riverside sectors inhabited by beavers along the Moson-Danube where we studied the beavers' nutrient preferences and habitat use based on the number and location of chewed trunks. We have identified positive preferences in the case of several tree and shrub species (*Corylus avellana*: 0.24 *Prunus padus*: 0.22 *Salix* sp.: 0.82) and we have determined the areas of these cultures that have been particularly affected. We found that 75% of the chewed trunks are within 10 metres of the riverbanks. As a result of our research, forests particularly sensitive to damage c

